Bio-Mineral Processing: A Suitable Approach

A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of

Master of Technology

in

Chemical Engineering

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2009

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by

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MASTER OF TECHNOLOGY (RESEARCH)

IN

CHEMICAL ENGINEERING

Under the esteemed guidance of

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NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA-769008.

ORISSA, INDIA.

2009

ABSTRACT

Bio-mineral processing is the generic term that describes the processing of metal containing ores, concentrator tailings, newly mined run-of-the-mine (ROM) material, and intermediate to high-grade ores using (micro-) biological technology. The application of bio catalyzed metal extraction has already been established for various metals in lab scale and the commercialization of processes for the extraction of the valuable gold, base metals like copper, zinc and cobalt from their high grade, sulfide ore bodies are over. For the lean grade ores and tailings, which are supposed to be the future resources, the processes are still in their infancy or confined in the laboratory scale. The potential of hetrotrophes for leaching/ beneficiation are yet to be fully exploited along with its major challenges in process design remain unresolved. The idea nurtured, expertise developed in the laboratories should find their way to get transformed in to feasible *technology*; then only the commercialization of such metal recovery processes from lean grade ores or overburdens will be shimmering as a reality.

The present work was an effort to bridge the gap between the lab-scale and commercial scale processes of bioleaching/beneficiation. For any process design and development at its transition between shake flask level and pilot plant level, one must understand the process mainly in two perspectives i.e. kinetics and optimization of design parameters; follows the techno economic feasibility. When a biological process is under consideration the characterization of different microbe present will also become important in optimizing the design parameters. Within the scope of the present work, the mentioned aspects have been studied in the context of leaching/beneficiation of three major and different base metals. The decision of choosing three different base metals was a conscious one for emancipating three different issues, conjectured with their present national and international status addressing them.

The present work emphasized on the characterization of a wide variety of heterotrophic micro organisms using iron ore beneficiation process and will contribute to the design database of bio-mineral processing using heterotrophes. In situ leaching with fungal strains such as *Aspergillus fumigatus, Penicillium citrinum and Aspergillus flavus* resulted in 7 %, 6 % and 17 %, removal of alumina, respectively. In situ leaching with *Aspergillus fumigatus, Penicillium citrinum and Aspergillus flavus* resulted in 8 %, 4 % and 16 %, removal of silica, respectively.

Bacillus polymyxa, Bacillus sphericus, and *Pseudomonas putida* ensured silica removal percentage of 10.6%, 5.3% and 20%, respectively. *Aspergillus flavus* and *Pseudomonas putida* were most efficient among all the bacterial and fungal strains used; ensuring iron beneficiation of about 3 % at the end of 10 days. The application of 2^4 level of full factorial design of experiments for zinc bioleaching using autotrophes manifested the screening of design parameters along with optimum parameter estimation. As a result of this diagnostic experiment, all the parameters considered in the present study including shaking speed were found to be statistically significant operating parameters. It also proposed the empirical model of the yield (zinc) as a function of major design parameters and the interactions among them using a statistical designing and analyzing software *MINITAB – 15*. The proposed empirical model is as follows,

$Y = 60.533 - 1902x_1 - 6.2x_2 - 3.723x_3 - 1.695x_4$, where Y is the yield of zinc.

The characterization of secondary metabolite excreted by heterotrophes, proposition of efficient kinetic mechanism for laterite ore and secondary metabolite interaction (a heterogeneous one for nickel extraction) adapting the shrinking core model were the valuable excerpts of the present study. The dissolution of nickel was ash layer diffusion controlled. From the apparent rate constants the effective diffusion coefficients were derived which are as follows,

Citric Acid $D_e = 1.98567\times10^{-9}$ cm^2/s

Oxalic Acid $D_e = 2.5907\times10^{-8}$ cm^2/s

Acetic Acid $D_e = 1.91904\times10^{-10}$ cm^2/s

The design and execution of model driven experiments to establish the kinetics and generation of design parameters extremely useful for modeling simulation and control of this microbial process based on first principle; were the meticulous efforts being taken in view of the future scale-up of the two-tank leaching process of chromites mine overburden using heterotrophes.

Keywords: *Aspergillus flavus, Pseudomonas putida*, laterite, heterotrophes, factorial design, secondary metabolite, shrinking core model.

National Institute of Technology

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CERTIFICATE

This is to certify that the thesis entitled "**Bio-Mineral Processing: A Suitable Approach**" submitted by **Ms. Shitarashmi Sahu**, in partial fulfillment of the requirements for the award of Master of Technology (Research) in Chemical Engineering, with specialization in '**Biotechnology'** at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter presented in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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ACKNOWLEDGEMENT

It is impossible to thank one and all in this book. A few however stand for me as I go on to complete this project. If words are considerable as symbols of approval and taken as acknowledgement then let the words play a heralding role in expressing my gratitude.

I would like to express my extreme sense of gratitude to **Dr. Madhushree Kundu**, Asst. professor NIT, Rourkela for her guidance throughout the work and her encouragement, positive support and wishes extended to me during the course of investigation.

I would also like to thank **Dr. S. K. Agrawal**, H.O.D, Dept of Chemical Engineering, NIT, Rourkela, for his support academically. A special thanks to Prof. **G. K. Roy,** Chemical Engineering Department, NIT, Rourkela, for their valuable advices and moral support.

I express my deepest sense of gratitude to **Mr L.B Shukla**, Scientist-G, Head of Department of Bio-minerals and **Dr. Nilotpala Pradhan**, IMMT, Bhubaneswar for their suggestion and guidance for my project.

I am also thankful to **Mr G. V Rao** for his help to collect the ore sample.

I am also thankful to **Mousumi Mishra,** IMMT, Bhubaneswar, for her support throughout the project work.

I am highly indebted to the authorities of NIT, Rourkela for providing me various facilities like library, computers and Internet, which have been very useful.

I express special thanks to all my friends, for being there whenever I needed them.
Thank you very much Tusar, Chaitu, Sonu, Arpita, Liku di, Pinaki.

Finally, I am forever indebted to my parents, sisters and brother for their understanding and encouragement when it was most required.

I dedicate this thesis to my family and friends.

Shitarashmi Sahu

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Nomenclature:

Symbol	Meaning
Y or R	Response variable (in chapter 3)
x	Factor
e	Random error
b	Model Coefficient
σ^2	Variance
m	Number of factorial runs
$\overline{Y}_C$	Average response of center points
$\overline{Y}_F$	Average response of factorial runs
N_C	Number of runs at center points
r_c	Unreacted core radius, **cm**
R	Initial particle radius, **cm** (in chapter 4)
τ	Time required for the particle to react completely, **sec**
t	Reaction time, **sec**
ρ_p	Molar density of solid reactant, **moles/cc**
α	Fraction of the reactant reacted
b	Stoichiometric coefficient
K_l	Mass transfer coefficient for liquid film, cm^2/sec
K_s	Reaction constant for surface reaction, sec^{-1}
D_e	Diffusion coefficient of ash layer, cm^2/sec

Chapter 1

INTRODUCTION TO BIO-MINERAL PROCESSING

Bio-mineral processing is the generic term that describes the processing of metal containing ores, concentrator tailings, newly mined run-of-the-mine (ROM) material, and intermediate to high-grade ores using (micro-) biological technology. Recently, the low-end recovery of metals from solid residues of low-grade ore, fly ash, galvanic sludge, or, in general, from industrial wastes also proclaims to be bio mineral processing . *Bio-mineral processing* through hydrometallurgical route demands an interdisciplinary contribution from the disciplines like geo-microbiology, microbial ecology, microbial biogeochemistry, hydrometallurgy and process engineering. When high-grade non-renewable mineral resources are being depleted necessitating the recovery of metals from low and lean grade ores, overburdens, and tailings; use of conventional techniques seems to be very expensive for the mineral processing industry due to its high energy and capital inputs accompanied by environmental burden. Biological leaching/beneficiation have the potential to address both economic and environmental issues associated with processing of metals. There are two broad categories of biologically assisted mineral degradation process. An ore or tailings from ore processing are either placed in a heap or dump, where it is irrigated or a finely milled mineral suspension placed in a stirred tank reactor where it is processed. In developing countries like India *bio-mineral processing* deserves greater

national significance, where there is a vast unexploited mineral potential and it can contribute to the economic and social development of these countries. There is a need of a national framework program of research and development aiming to stimulate synergies between research laboratories and end-users favoring the emergence of innovative and sustainable biohydrometallurgical processes; which meets the need of the present without compromising with the ability of the future generations to meet their own needs.

1.1. PARTICIPATION OF MICROORGANISMS IN MINERAL PROCESSING

In bio-mineral processing, the natural ability of micro organisms to interact with metal ion has been effectively utilized. It was not until 1947 that these phenomena were attributed to bacteria. Once identified, however, rapid steps were taken to commercialize the processes. Commercial application of bacterial leaching began in the late 1950s at the Kennecott Utah Copper Company's Bingham Canyon Mine near Salt Lake City, Utah where it was observed that blue copper-containing solutions were running out of waste piles that contained copper sulfide minerals, something that should not have happened in the absence of powerful oxidizing agents and acid. Biological activity related to mineral dissolution has long been known to occur in nature and in fact is primarily responsible for acid mine drainage (AMD). Several types of autotrophic and heterotrophic bacteria, fungi, yeasts and algae are acting as biocatalyst in mineral beneficiation and bioleaching processes. Almost all microorganisms interact with metals either directly or indirectly. Bioleaching is the first and primary component of bio-geotechnology and it is the first step of biohydrometallurgy leading to the formation of lixiviant, henceforth, the pure metal can be extracted by different techniques like electro winning. Leaching can also be explained as the solubilization of one or more components of a complex solid by contact with a liquid phase. Bio-beneficiation refers to removal of undesirable mineral components from an ore through interaction with micro organism which bring about their selective removal and thereby, enriching the desire mineral constituent in the solid ore matrix mediated by a number of surface chemical and physiochemical phenomenon (Ex: Bio- desulfurization of coals, and Bio-beneficiation of iron ores). The potential benefits of bioleaching process for treating ores and concentrates can be summarized as follows:

- Low capital investment.
- A reduced amount of energy consumption.
- The relative absence of air, water, land pollution.
- The process can be applied for low/ complex ores.
- Degradation of variety of mineral forms.
- Selective leaching possible, one mineral solubilize while the other remains insoluble.
- Low energy requirement and operating costs as compared to conventional process for recovery of metals from low grade ores.
- Zero discharge, i.e. recycling of effluents.
- The process is site specific.
- The microorganisms are available indigenously.
- A reduced amount of technical sophistication.
- Simple technology which is easy to operate and maintain in heap, dump or bioreactors (single or multiple).
- The process is safe and conforms to nature.
- Free from gaseous and dust emission. Can handle variety of simple and complex materials of low grade as well as concentrates.
- The processes are more environmentally friendly than traditional extraction methods, for the company this can translate into profit. Since necessary limiting of sulfur dioxide emissions during smelting is expensive.
- Application of most useful is desulphurization of coal for burning is free of sulphur and further pollution problem avoidable.
- A reduced amount of process control.
- Operate at ambient temperature and normal atmospheric pressure.
- Suitable for less developed countries as it eliminates the need for some costly and imported heavy mining equipment.

There are also some disadvantages of the process:

- The bacterial leaching process is very slow compared to smelting.

- This brings less profit as well as introducing a significant delay in case flow for new plants.
- Toxic chemicals are sometimes produced in this process.
- *In-situ* application still under development.
- Control difficulties.
- More possible for acid producing minerals and not acid consuming minerals.

Microorganisms thrive naturally in various environmental conditions exhibiting diverse characteristics that can be specially exploited to our needs and make them suitable for microbial leaching process. The microorganisms utilize the metal ions as a part of their life cycle and convert it to soluble form. Although micro-organisms cannot destroy metals (they are not alchemists!) they can alter their chemical properties via a surprising array of mechanisms (fig. 1.1). In some cases these processes involve highly specific biochemical pathways that have evolved to protect the microbial cell from toxic heavy metals. In some cases, microbes can produce new mineral phases via nonspecific mechanisms that result in the entrapment of toxic metals within soils or sediments. Other mechanisms of potential commercial importance rely on the production of biogenic ligands that can complex metals, resulting in their mobilization from contaminated soils and low grade ores.

1.2. COMMERCIAL BIO-MINERAL PROCESSING AND ITS STATUS

There are two broad categories of biologically assisted mineral degradation process. An ore or tailings from ore processing are either placed in a heap or dump, where it is irrigated or a finely milled mineral suspension placed in a stirred tank reactor where it is processed. Stirred tank processes equipped with pH and temperature control devices use highly aerated continuous flow reactors. Finely grinded ore is mixed with inorganic nutrients in the form of ammonia or phosphate containing fertilizers irrespective of the fact that autotrophic or heterotrophic microbes are catalyzing the process. Mineral decomposition takes only a few days in stirred tank reactors compared with weeks or months in heap/dump leaching. The tank leaching remained restricted so far to high value mineral or mineral concentrates owing to its limitation in handling higher pulp density and considerably higher fixed as well as running costs than heap leaching. The growth selectivity and steadiness of the microbial ecosystem in the bioreactors compared to the

diversity of the natural environment which prevails in heap/dump leaching offers the privileged condition for better yield in bioreactors. In juxtaposition, it is not prudent to assume that the medium or industrial scale bioreactors will contain the same biological composition as in the best operating conditions at lab scale. Moreover the best operating conditions are generally the result of months of continuous growth at lab scale which are not completely reproducible at industrial level. The heap leaching is suitable for treatment of low grade ore bodies and overburdens because it is easy to construct and operate. But inability in maintaining constant pH gradient, temperature, selective microbial colony, homogeneous mineral and nutrient composition, hence the desired reaction rate is the major roadblock towards the commercialization of such processes for many instances. Gold and copper from their sulfide ores are commercially produced in Uzbekistan, Australia, Greece, and Chile through the bio-oxidation process using Gold Fields proprietary BIOX and BIOCOP processes supplied by Biomin technologies, South Africa, and BHP Bilton. Kasese Plant in Uganda uses BRGM (Bureau de Recherches Géologiques et Minières) technology for the recovery of cobaltiferous pyrite. A major challenge is to find a suitable match between an ore body and a suitable bioleaching/beneficiation technology. For instance the technology of recovery of nickel from its sulfide ore using BIONIC process has been theoretically tested, but any ore body of suitable concentration and size is yet to be identified to allow economic recovery at the current nickel price. Commercial heap leaching units are in operation over quite some time for the recovery of copper mainly from their sulfide ores in countries like Chile, USA, and Australia. Copper recovery from bioleaching accounts for about 25% of the world copper production. A major multidisciplinary and multi-institutional Chilean project on bacterial leaching of copper ore is considered a landmark in bioleaching technology. The project, funded by the Chilean government and the United Nations Development Program – UNDP, started in 1985.

India has the distinction that the deepest ancient mines in the world for gold come from the Maski region of Karnataka with carbon dates from the mid 1st millennium BC. Technology developed by K A Natarajan and co-workers at the Indian Institute of Science is being applied at the Hutti Gold Mines, Karnataka, India for extraction of gold.

The sulfide ores have been the major source of various base metals like nickel to date, however the lateritic ores have been estimated to constitute about 73% of the known nickel

reserves of the world. No commercialized process to extract nickel from this low grade ore bodies by biohydrometallurgical processes has been reported so far; either by autotrophs or by heterotrophs. To extract nickel from the chromite overburden, generated during mining of chromite ore in Sukinda Valley, Orissa, India, a non-biological process route has been developed jointly by the Department of Mines and the Council for Scientific and Industrial Research (CSIR). To verify the critical parameters of the process, a 10 tonnes per day ore throughput pilot plant has been set up at Institute of Minerals and Materials Technology (IMMT), Bhubaneswar at a cost of Rs10.5 crore; jointly by HZL and CSIR.

The application of bio catalyzed metal extraction has already been established for various metals in lab scale and the commercialization of processes for the extraction of the valuable gold, base metals like copper, zinc and cobalt from their high grade, sulfide ore bodies are over. For the lean grade ores and tailings, which are supposed to be the future resources, the processes are still in their infancy or confined in the laboratory scale. The potential of heterotrophs for leaching/ beneficiation are yet to be fully exploited along with its major challenges in process design remain unresolved. The idea nurtured, expertise developed in the laboratories should find their way to get transformed in to feasible *technology*; then only the commercialization of such metal recovery processes from lean grade ores or overburdens will be shimmering as a reality.

1.3. OBJECTIVE

For any process design and development at its transition between shake flask level and pilot plant level, one must understand the process mainly in two perspectives i.e. kinetics and optimization of design parameters; follows the techno economic feasibility. When a biological process is under consideration the characterization of different microbe present will also become important in optimizing the design parameters. The present work was an effort to bridge the gap between the lab-scale and commercial scale processes of bioleaching/beneficiation in the framework of the following perspectives:

1. Characterization of microorganisms
2. Optimization of parameters
3. Evaluation of suitable kinetics

Within the scope of the present work, the mentioned aspects have been studied in the context of leaching/beneficiation of three major and different base metals. The decision of choosing three different base metals was a conscious one for emancipating three different issues, conjectured with their present national and international status addressing them. The present work also attempted to explore the potential of the selected approaches for three different bio catalyzed processes, namely, beneficiation process using heterotrophic microorganisms, leaching process using autotrophs and leaching process using secondary metabolic acids excreted by heterotrophic microorganisms.

1.4. ORGANIZATION OF THE THESIS

In view of the aforesaid objectivities, present work is divided in to three broad categories.

Part 1: Characterization of different heterotrophs: In the context of bio-beneficiation process of iron ore.

Part 2: Optimization of different parameters in bioleaching using factorial design approach: In the context of Zinc sulfide leaching using *Thiobacillus ferrooxidans.*

Part 3: Proposition suitable dissolution kinetics for the heterogeneous reaction: Reaction of nickel laterite ore in different secondary metabolic acids produced by heterotrophic microorganisms.

First chapter renders an overview of the bio-mineral processing, the role of microbes in it, the present national and international state of art of the commercialization of *bio- mineral processing,* the objective of the proposed work and organization of the thesis. The second chapter emphasized on the characterization of a wide variety of heterotrophic micro organisms using iron ore beneficiation process and will contribute to the design database of bio-mineral processing using heterotrophs. In the chapter three, the application of 2^4 level of full factorial design of experiments for zinc bioleaching using autotrophs manifested the screening of design parameters along with optimum parameter estimation. It also proposed the empirical model of the yield (zinc) as a function of major design parameters and the interactions among them using a statistical designing and analyzing software *MINITAB – 15*. The characterization of secondary

metabolite excreted by heterotrophs and proposition of efficient kinetic mechanism for laterite ore and secondary metabolite interaction (a heterogeneous one for nickel extraction) adapting the shrinking core model; were the excerpts of chapter four. In an ending note, the fifth chapter concludes with recommendation of future research initiatives.

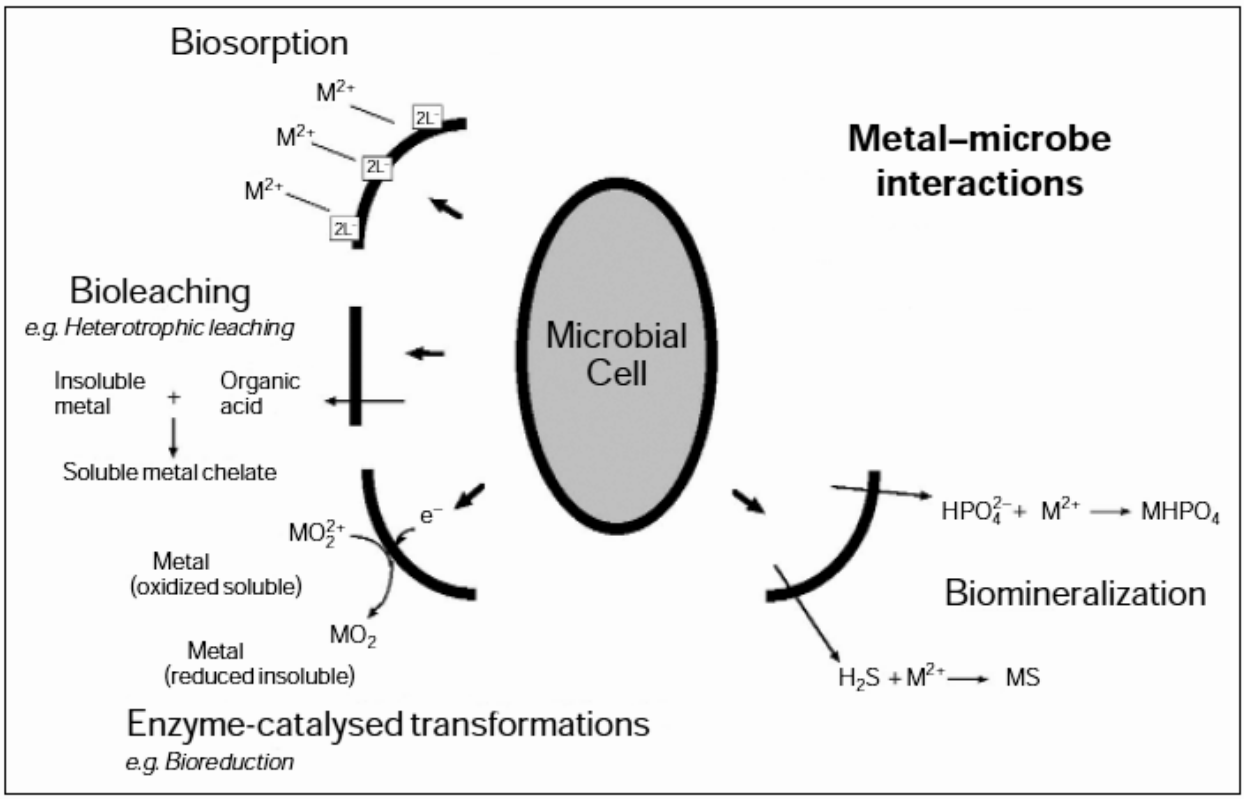

Figure 1.1 Mechanisms of metal–microbe interaction.

Chapter 2

CHARACTERIZATION OF DIFFERENT HETEROTROPHS: IN THE CONTEXT OF BIO-BENEFICIATION PROCESS OF IRON ORE.

2.1. INTRODUCTION

The second chapter emphasized on the characterization of a wide variety of heterotrophic micro organisms using iron ore beneficiation process as a case study and exhaustive characterization of a wide variety of them will contribute to the design database of bio-mineral processing using heterotrophs. This chapter presents an exhaustive documentation of resources of iron ore in India, important ores of iron, need of beneficiation of iron ore in connection to its use in steel making.

Iron ores are rocks and minerals from which metallic iron can be economically extracted. The ores are usually rich in iron oxides and vary in color from dark grey, bright yellow, deep purple, to rusty red. The iron itself is usually found in the form of magnetite (Fe_3O_4), hematite (Fe_2O_3), goethite, limonite or siderite. Hematite is also known as "natural ore". The name refers to the early years of mining, when certain hematite ores contained 66% iron and could be fed directly into iron making blast furnaces. Iron ore is the raw material used to make pig iron, which is one of the main raw materials to make steel. 98% of the mined iron ore is used to make steel.

Pure iron is virtually unknown on the surface of the Earth except as Fe-Ni alloys from meteorites and very rare forms of deep mantle xenoliths. Therefore, all sources of iron used by human industry exploit iron oxide minerals and the primary form which is used in industry being hematite. However, in some situations, more inferior iron ore sources have been used by industrialized societies when access to high-grade hematite ore was not available. This includes utilization of taconite and goethite or bog ore. Magnetite is often used because it is magnetic and hence easily liberated from the gangue minerals. Hematite iron ore deposits are currently exploited on all continents, with the largest intensity in South America, Australia and Asia. Hematite iron is typically rarer than magnetite bearing BIF (banded iron formation) or other rocks which form its main source or protolith rock, but it is considerably cheaper and easier to beneficiate the hematite ores and requires considerably less energy to crush and grind. Hematite ores however can contain significantly higher concentrations of penalty elements, typically being higher in phosphorus, water content and aluminium (clays within pisolites).

The total recoverable reserves of iron ore in India are about 9,602 million tones of hematite and 3,408 million tones of magnetite. Madhya Pradesh, Karnataka, Bihar, Orissa, Goa, Maharashtra, Andhra Pradesh, Kerala, Rajasthan and Tamil Nadu are the principal Indian producers of iron ore. Fig. 2.1 shows the Indian location of iron ores. Low grade iron ores are available in Goa (Fe content: 48 -56 %), Jharkand and Orissa.

The production of iron ore in India was about 206.45 million tonnes in 2007-08 registered an increase of 10% over the previous year. The value of metallic minerals in 2007-08 at Rs.24,038 crores increased by about 31% over 2006-2007. Among the principal metallic minerals, iron ore contributed Rs.18, 495 crores or 76.9% (Annual Report 2008-2009, Ministry of Mines, India) of Indian mineral economy. World production averages one billion metric tons of raw ore annually. The world's largest producer of iron ore is the Brazilian mining corporation Vale, followed by Anglo-Australian companies BHP Billiton and Rio Tinto Group. An Australian supplier, Fortescue Metals Group Ltd may eventually bring Australia's production to second in the world. World consumption of iron ore grows 10% per annum on average with the main consumers being China, Japan, Korea, the United States and the European Union. China is currently the largest consumer of iron ore and to be the world's largest steel producing country.

Iron ore reserves at present seem quite vast, but it is being apprehended that the mathematics of continual exponential increase in consumption can prove this resource to be quite finite. For instance, Lester Brown of the World Watch Institute has suggested iron ore could run out within 64 years based on an *extremely conservative* extrapolation of 2% growth per year (Burgstaller and Schinner, 1993). During the iron ore mining 15-20% of the wash is produced as fines containing 50-80% Fe and is discarded as tailing and slime. These fines cannot be utilized in their native form as they contain high alumina and silica content but can be re-utilized by bringing down their alumina and silica percentage. Utilization of low grade iron ore (below 60% Fe) resources, not being utilized currently, is crucial to ensure optimal resource utilization and maintaining future profit margins in steel making industries.

2.1.1. Biobeneficiation

Iron ore generally contains alumina, silica, sulphur and phosphorous as impurities. High percentage of alumina and silica present in iron ore has adverse effect on reducibility, coke rate, productivity and blast furnace operation, for steel making. Bio beneficiation may be one of the most eco-friendly, promising and revolutionary solutions in this regard. Bio-beneficiation refers to removal of undesirable mineral components from an ore through interaction with micro organism which bring about their selective removal by bio leaching process and thereby, enriching the desired mineral constituent in the solid ore matrix mediated by a number of surface chemical and physiochemical phenomenon, which are as follows,

- Alteration of the surface chemistry of minerals
- Generation of surface active chemicals
- Selective dissolution of mineral phases in an ore matrix and
- Sorption, accumulation and precipitation of ions and compounds.

Several types of autotrophic and heterotrophic bacteria, fungi, yeasts and algae may be involved in mineral beneficiation. The micro organisms have been reported to solubilise different alumina silica, titanium, copper, sulphur compounds found in nature. Heterotrophic microorganisms degrade the alumino silicates by using the soluble low molecular weight metabolites, organic acids and amino acids etc. These metabolites can dissolve metals from

minerals by displacement of metal ions from the ore matrix by hydrogen ion and formation of soluble metal complexes and chelators. Fig. 2.2 represents the mechanism of heterotrophic bioleaching of alumina and silica, hence, bio-beneficiation of iron.

In the present study, the heterotrophic bacteria and fungus were used for removal of alumina and silica from iron ore so that the iron content of the concerned ore increases. Increase in iron percentage in iron ore increases its value and make it acceptable to iron making by existing metallurgical techniques. The ore beneficiation is carried out by the secondary metabolites produced by these heterotrophic micro organisms (Cameselle et *al.,* 2003, Ehrlich, 2001). Since ores are not sterile and cannot be sterilized on a commercial scale, heterotrophic leaching poses some process development and design challenges that autotrophic leaching does not (Ehrlich, 2001). This can be carried out via *in situ* leaching or *two tank* leaching (Groudev, 1987, Cameselle et *al.*, 2003).

The present investigation demonstrated the bio-beneficiation process and its optimization for Guali iron ore using *Bacillus polymyxa, Bacillus spharicus, Pseudomonas putida, Aspergillus fumigatus, Penicillium citrinum,* and *Aspergillus flavus.* In accordance with the wet chemical and instrumental technique (XRF), the iron ore sample obtained from Guali mines, Orissa, India was found to contain about 76-78 % Fe_2O_3, apart from Al_2O_3, SiO_2, P_2O_5, and TiO_2 as undesirable components. The different parameters influencing the growth of the micro organism concerned were investigated for ensuring optimal growth. It becomes important to determine the onset of the stationary phase as the production of secondary metabolites occurring at this phase. Hence, experimental growth studies were conducted to determine the commencement of the stationary phase for bacterial and fungal strain. The tentative flow chart for the beneficiation process is given in fig. 2.3.

2.2. REVIEW OF LITRATURE

The biological treatment of ores to remove contaminants, often referred to biobeneficiation (Jain and Sharma, 2004) is another variant of the chemical processing. In such a process the microorganisms produce, as a consequence of their metabolisms, a chemical bio product (mineral acid, organic acid polymer, enzyme, chelating agent, etc.). The chemical bio

product, in turn attack the gangue materials contain in the ore, dissolving them and thus producing their selective removal (Jain and Sharma, 2004). The extraction of iron is done from the solid part and aluminium from the liquid or leach liquor by solvent extraction which followed precipitation. (Mishra et *al.,* 2009). The iron ore is not suitable in iron and steel making due to the presence of higher amount of gangue constituents. Several beneficiation techniques have been tried from time to time to reduce the gangue constituents so that the beneficiated products could be effectively used for iron and steel making (Das et *al.,* 1992, Pradip, 1994, Prakash et *al.,* 1999).

Impurities present in iron ores comprise both metallic and non-metallic components. Usually, siliceous gangue consists of larger proportion of alumina in the form of clay and laterite along with varying amounts of undesirable constituents such as phosphorous, sulphur, titanium, copper and arsenic. Many microorganisms have been reported to solubilise different alumino-silicate compounds found in nature. In silicates, silicon is usually surrounded by four oxygen atoms in tetrahedral fashion whereas aluminium in alumino silicates is coordinated with oxygen in tetrahedral or octahedral fashion, depending upon the mineral (Tan, 1986). In minerals, these units are arranged in bi- or tri-layers separated by water layers of variable thickness into which other polar molecules, including some organic molecules can enter. This type of structure makes them susceptible for weathering by microorganisms. Si–O bonds of silloxanes linkages (Si–O–Si) in silicates and alumino silicates are very strong, whereas Al–O bonds are somewhat weaker. Thus Si–O bonds are relatively resistant to acid hydrolysis (Karavaiko et *al.,* 1985), unlike Al–O bonds. Bacteria and fungi solubilise silica and silicates by forming chelators, exo-polysaccharides, acids or bases. Both *Aspergillus* sp. and *Bacillus* sp. are known to be involved in the leaching and beneficiation processes of silicate ores and minerals (Karavaiko et *al*., 1980 and Avakyan et *al.*, 1986).

During metabolism, microorganisms convert glucose or other carbohydrates into variety of products, including organic acids. Bio-leaching processes are mediated due to the chemical attack by the extracted organic acids on the ores. Acids usually have dual effect of increasing metal dissolution by lowering the pH and increasing the load of soluble metals by complexing/chelating into soluble organo-metallic complexes (Burgstaller and Schinner, 1993). Citric acid is a tri-carboxylic and one hydroxyl group as possible donor of protons (H^+). When

aluminium cations Al^{+3} are present in system and citric acid is fully dissociated in aqueous solution, a complexation reaction may take place (Ghorbani et *al.*, 2007) in the following way,

$$C_6H_8O_7 \leftrightarrow (C_6H_8O_7)^{3-} + 3H^{+}(pKa_3 = 6.39) \quad (2.1)$$

$$(C_6H_8O_7)^{3-} + Al^{3+} \leftrightarrow Al(C_6H_5O_7) \quad (Aluminium\ citrate\ complex) \quad (2.2)$$

Similarly, oxalic acid contains two carboxylic groups (pKa_1=1.20 and pKa_2=4.20 at 25°C); so the possible complexes of aluminium cation with oxalate anion are

$$C_2H_4O_5 \leftrightarrow (C_2HO_4)^{1-} + H^{+}(pKa_1 = 1.20) \quad (2.3)$$

$$3(C_2HO_4)^{1-} + Al^{3+} \leftrightarrow Al(C_2HO_4)_3 \ (Aluminium\ oxalate\ complex) \quad (2.4)$$

$$C_2H_2O_4 \leftrightarrow (C_2O_4)^{1-} + 2H^{+}(pKa_2 = 4.20) \quad (2.5)$$

$$3(C_2O_4)^{1-} + 2Al^{3+} \leftrightarrow Al_2(C_2HO_4)_3\ (Aluminium\ oxalate\ complex) \quad (2.6)$$

According to the open literature the microorganisms are likely able to mobilize metals by (i) formation of organic acid, (ii) oxidation- reduction reactions, (iii) extraction by complexing agents, (iv) chelate formation with the cations (Aluminium and Silicon). Although the use of different microorganisms in ore leaching is well-established, use of microorganism to reduce alumina and silica from ore has been attempted in very few investigations (Pradhan et *al*., 2006; Natarajan and Deo et *al.*, 2000). In view of this, the present chapter is devoted to the bio-beneficiation process development of Guali iron ore using a wide variety of heterotrophs and characterization of some of the selected bacterial and fungal strengths.

2.3. MATERIALS AND METHODS

2.3.1. Sample

Iron ore sample was obtained from Guali iron ore mines, India. The sample was analyzed by wet chemical and instrumental techniques (XRF) (Vogel, 1978). The sample was collected, ground, dried in a hot air oven (105°C) and analyzed by standard methods (Vogel, 1978).

2.3.2. Chemical Analysis of Iron Ore

One gram of sample was digested with help of 50 ml of concentrated HCL in beaker. The beaker was kept over a hot plate and boiled for 4 hours. After complete digestion of the samples, the liquid was filtered into separate 250 ml volumetric flasks by using Whatman 41 filter papers. The residue was washed thoroughly by distilled water & finally the volume was made up to the mark. The solution after suitable dilutions was used for quantitative estimation of different metals with the help of Perkin Elmer Atomic Absorption Spectrophotometer.

2.3.3. Microorganisms

Pseudomonas putida (NCIM-2650) and *Aspergillus flavus* (NCIM-554) were used for this study. Both these strains are obtained from National Collection of Industrial Microorganisms **(**NCIM**)**, Pune and other strains were collected from department of Bio-minerals, Institute of Minerals and Materials Technology (IMMT), Bhubaneswar. *Bacillus polymyxa, Bacillus sphericus, Pseudomonas putida, Aspergillus fumigatus, Penicillium citrinum, Aspergillus flavus* were used for this study. Bacterial strains were maintained on nutrient agar and fungal strains on potato dextrose agar slants, respectively. Bromfield medium containing (g/L) sucrose 20, yeast extract 1, K_2HPO_4 0.25, NH_4SO_4 0.25, $MgSO_4$ 0.75, sodium bisphosphate 0.30, and having pH of 6.8 ± 0.2 was used for inoculation and growth of *the* aforementioned bacterial and fungal strains. Figs. 2.4-2.9 show the colony morphology of different strains.

2.3.4. Characterization of Parameters Affecting the Growth of Microbes

2.3.4.1. Studying effect of media

Both bacteria and fungi were inoculated in four different 250 ml conical flasks containing MSM and BM media (Tables 2.1& 2.2) and kept in a shaker incubator at 33^0C at 100rpm for 7 days.

2.3.4.2. Studying the effect of pH

Five different pHs (5, 5.5, 6, 6.5, and 7) were maintained in 5 different flasks with BM media for fungi and MSM media for bacteria. Fungal strain had shown better growth in BM media and bacterial strain in MSM media. Both *Pseudomonas putida* and *Aspergillus fumigates* were chosen to demonstrate growth study and its influencing parameters and were inoculated and incubated as above for 7 days.

2.3.4.3. Utilization of carbon source

To study the utilization of carbon source (both sucrose and glucose) a titration method using Fehling's reagent was used. For this purpose the bacterial and fungal strains were inoculated in BM with both sucrose and glucose as carbon sources and incubated as above. For the estimation of unutilized carbon source in the media the procedure followed were:

2.3.4.3.1. Estimation of reducing sugar in culture filtrate

Reagent Preparation:

i. Fehling's A – 34.65 of $CuSO_4$ was dissolved in distilled water and the volume was made up to 500ml.
ii. Fehling's B – 125g of potassium hydroxide and 173 g potassium sodium tartarate were dissolved in distilled water and the volume was made up to 500ml.
iii. Standard glucose solution – 10 g glucose was dissolved in 100ml distilled water.
iv. Unknown glucose solution of culture filtrate was collected.

Procedure:

5 ml of Fehling's A & 5 ml of Fehling's B were added in a conical flask. Then 40 ml of distilled water was added to it. After that, the mixture was heated over the flame. The standard glucose solution was taken in a burette. The titration was done with drop wise addition of sugar solution into boiling Fehling's solution. Titration was continued till brick red colour appeared

(after total disappearance of blue colours of the mixture). Titration was repeated to get 3 concurrent readings. The amount of glucose solution required to titrate a fixed volume of Fehling's solution was determined. Same procedure was repeated with unknown glucose solution taken in burette. After comparison with standard glucose solution the concentration of glucose in unknown solution was determined.

Calculation:

$$\text{Strength of unknown glucose solution} = \frac{\text{Strength of standard glucose solution} \times \text{Volume of standard solution}}{\text{Volume of unknown solution}}$$

2.3.5. Bio Beneficiation

2.3.5.1. Beneficiation with bacteria

In situ leaching experiments to remove alumina and silica with *Bacillus polymyxa, Bacillus sphericus, and Pseudomonas putida* were carried out in 100 ml of Bromfield medium in 250 ml Erlenmeyer flask under sterile conditions at a pulp density, temperature and agitation speed of 5 %, 35 °C and 150 rpm, respectively. Inoculation was done with 10% inoculums (v/v) containing 1×10^8 cfu/ml of *Bacillus polymyxa, Bacillus sphericus, Pseudomonas putida* and incubation time was 10 days. At the end of experiments, solid residue was separated by filtration through medium fast filter paper, dried in hot air oven and analyzed for Al and Fe. The pH of the filtrate was determined with help of pH meter.

2.3.5.2. Beneficiation with fungus

Aspergillus fumigatus, Penicillium citrinum and *Aspergillus flavus* were the fungus used for in-situ leaching of alumina and silica present in the ore. It was done in 100 ml of Bromfield medium under sterile conditions. Iron ore sample was added at 5% pulp density (w/v). Inoculation was done with 10% inoculum (v/v) containing 1×10^6 spores/ml of the aforesaid spores. Incubation temperature and agitation speed were 35 °C and 150 rpm, respectively, and incubation time was 10 days. At the end of experiments, solid residue was separated by filtration through medium fast filter paper, dried in hot air oven and analyzed for Al and Fe. The pH of the filtrate was determined with help of pH meter.

2.3.6. Growth Study

As *Pseudomonas putida* and *Aspergillus flavous* have shown encouraging results in leaching the undesirable components like alumina and silica, we preferred to study the growth characteristics of those strains.

2.3.6.1. Kinetic growth study of bacteria

The bacterium was cultured with 200ml nutrient broth in 250ml standard Erlenmeyer shake flask in an incubator shaker at 100 rpm and 30^0**C.** To avoid the lag phase the culture was kept overnight around 12 hrs. For the estimation of biomass, the absorbance of the media was studied with respect to time with the help of Jasco V-530 UV/VIS spectrophotometer. The absorbance values were taken at a time interval of 30 minutes until there was no change in absorbance value which indicated the on-set of stationary phase. Vertexing has been done before every reading in order to get homogenized sample for accurate absorbance values.

2.3.6.2. Biomass growth study of fungus

The absorbance study for fungus is not possible as they form spores (mat like) in the broth. So the biomass weight for the fungus was directly measured. For this purpose the Bromfield media in 6 different flasks were kept in the shaker to allow them for growth after inoculation. Each day one flask was taken and filtered for the biomass by using filter paper. The filter paper with biomass was kept in hot air oven at temp 90^0C. After the filter paper has been dried completely, the weight of that filter paper was determined, hence, the dry weight of the fungal biomass.

2.3.7. Effect of Growth on Media pH

For this purpose, Bromfield media at five different pH values (7, 6.5, 6, 5.5, and 5) in five different flasks were taken. After inoculation, those were kept for five days in shaker. After incubation the dry weights of the biomass was determined by using the same method mentioned in section 3.6.2.

2.4. RESULTS AND DISCUSSION

2.4.1. Analysis of Iron Ore

Complete chemical analysis of Guali iron ore revealed that the sample on an average contained 53 % total (Fe) iron and alumina concentration in the sample was quite high, besides this, the ratio of SiO_2/Al_2O_3 in the original ore was also very high contradicting to blast furnace chemistry (Table 2.3). An iron ore containing more than 58-60 % iron is considered suitable for steel making by existing technology. All the facts accelerated the endeavor to make this pristine ore to be suitable for steel making by using microorganisms.

2.4.2. Effect of Media on Growth

While studying the effect of media on bacterial and fungal strains, *Pseudomonas putida* showed significant growth in MSM whereas *Aspergillus fumigates* had shown better growth in BM. Biomass produced by *Pseudomonas putida* in BM and MSM were 1.2gm/200ml and 1.8gm/200ml, respectively. *Aspergillus fumigates* produced 5.4gm/200ml of biomass in BM and 3.7gm/200ml of biomass in MSM. Fig. 2.10 shows the growth patterns of both bacteria and fungus in two different media.

2.4.3. Effect of pH on Growth

The effect of initial pH of the media on microbial growth as revealed in fig. 2.11 shows that both *Pseudomonas putida* and *Aspergillus fumigatus* have followed same growth pattern in different initial pH conditions. Both the strains had shown better biomass production, when the incubation got started at 6.5 pH range.

2.4.4. Utilization of Carbon Source

Microorganisms utilized both carbon sources effectively with a little variation. *Aspergillus fumigatus* utilized the carbon sources more rapidly as compared to *Pseudomonas putida*. The consumption of sucrose and glucose in the first day of incubation by *A.fumigatus* was 8.2 gms and 8.4 gms, respectively; while that was 6.3 gms and 6.8 gms, respectively by *P.putida*. Consumption of glucose is little bit more in comparison to sucrose as it is a readily

utilizable energy source for these micro organisms. When the utilization of carbon source is more the microbial growth is also better. Hence, both the strains had shown a little bit better growth in glucose in comparison to sucrose. Figs. 2.12 & 2.13 reflect the residual sucrose and glucose in the media, hence the utilization pattern of those carbon sources by *Aspergillus fumigatus* and *Pseudomonas putida*, respectively.

2.4.5. Bioleaching Experiments

The solubilizing action by microorganisms may involve the cleavage of Si–O–Si or Al–O framework bonds, or the removal of cations from the crystal lattice of aluminosilicate causing the subsequent collapse of silicate lattice structure. In situ leaching with fungal strains such as *Aspergillus fumigatus, Penicillium citrinum and Aspergillus flavus* resulted in 7 %, 6 % and 17 %, removal of alumina, respectively, (fig. 2.14). The bacterial strains like *Bacillus polymyxa, Bacillus sphericus,* and *Pseudomonas putida* ensured an alumina removal of 15 %, 8 % and 17 %, respectively, (Fig. 2.14). In situ leaching with *Aspergillus fumigatus, Penicillium citrinum and Aspergillus flavus* resulted in 8 %, 4 % and 16 %, removal of silica, respectively, (fig. 2.15). *Bacillus polymyxa, Bacillus sphericus,* and *Pseudomonas putida* ensured silica removal percentage of 10.6%, 5.3% and 20%, respectively, (fig. 2.15). According to the XRF studies, *Aspergillus flavus* and *Pseudomonas putida* catalyzed the increment of Fe_2O_3 percentage in the treated iron ore by 3 % at the end of 10 days by their selective removal of alumina and silica. This study also revealed the simultaneous iron removal by microorganisms like *Bacillus sphericus* and *Penicillium citrinum* while removing silica and alumina. Iron ore beneficiation results involving various microorganisms are shown in fig. 2.16.

2.4.6. Growth Study

2.4.6.1. Bacterial growth study

Pseudomonas putida had shown a decent growth rate when incubated in nutrient media. In the growth curve, the exponential phase is consistent with a slope ≈ 0.2 units/hr (fig. 2.17). The absorbance pattern was recorded after 12hrs in order to omit the initial lag phase. The stationary phase reached nearly after 24hrs from the inoculation.

2.4.6.2. Fungal growth study

Aspergillus flavous has shown a very slow growth rate when compared to bacterial strain *Pseudomonas putida*. The growth curve reached its exponential phase nearly after 24hrs. It took five days to reach the stationary phase (fig. 2.18).

2.4.7. Biomass Growth and pH

It has been observed that the pH of the media is continuously decreasing with the biomass growth (fig. 2.19). The neutral pH at the beginning for all the beneficiation experiments with fungi and bacterial strains turned to acidic ones at the end.

2.5. CONCLUSIONS

The choice of media, effect of initial pH at the starting of incubation period, utilization of carbon sources are very important information regarding the growth and sustenance of microbes in the concerned operation. Some salient features coming out of this study are summarized as follows:

Media effect on the growth of microbial biomass revealed that *Pseudomonas putida* had better growth in MSM and *Aspergillus fumigates* in BM media. The pH of the medium was declining with the growth of the micro organisms. But both the strains had shown better biomass production, when the incubation got started at 6.5 pH. While monitoring the residual sugars (glucose, sucrose); it was evident that *Aspergillus fumigates* utilized more sugars than *Pseudomonas putida* and both the microorganisms individually showed a reasonable consumption of glucose and sucrose. For both strains a cheap carbohydrate source like sucrose can be used in place of glucose, which may contribute to the overall economy of the process.

The alumina present in the iron ore poses problem of handling high viscous solution in blast furnace operation. In situ leaching of Guali iron ore by *Aspergillus fumigatus, Penicillium citrinum,* and *Aspergillus flavus* removed about 7 %, 6 % and 17 % of alumina in 10 days at 5% pulp density. *Bacillus polymyxa, Bacillus sphericus,* and *Pseudomonas putida*, removed about 15 %, 8 % and 17 % of alumina in 10 days at 5% pulp density. Apart from alumina, 10.6 %, 5.3 %

and 20 % silica were removed with the help of Bacillus *polymyxa, Bacillus sphericus,* and *Pseudomonas putida,* respectively, in 10 days at 5% pulp density. *Aspergillus fumigatus, Penicillium citrinum* and *Aspergillus flavus* removed 8 %, 4 %, and 16 % silica, respectively, at the end of 10 days with 5% pulp density. *Aspergillus flavus* and *Pseudomonas putida* were most efficient among all the bacterial and fungal strains used; ensuring iron beneficiation of about 3 % at the end of 10 days. *Bacillus sphericus* and *Penicillium citrinum* leach out iron simultaneously with alumina and silica. From the growth studies of bacterial and fungal strains, it can be concluded that leaching with heterotrophic bacteria is less time consuming or faster than in comparison with fungal strains. The earlier onset of stationary phase for the bacterial strain in comparison to fungal strain supposed to be responsible for accelerated leaching out of alumina and silica from the iron ore. *Pseudomonas putida* is a potential microorganism for economic leaching, hence, beneficiation. From the aforesaid facts, the bio-beneficiation of iron ore by incrementing iron percentage and removing undesirable silica and alumina, from the pristine ore, hence altering the alumina to silica ratio suitable for blast furnace operation seemed to be a promising and eco-friendly alternative.

Table 2.1. Mineral Salt Medium Composition

S. No.	Constituents	Amount (g/L)
1	KNO3	3
2	KH2PO4	0.36
3	MgSO4	0.5
4	Carbon source	10%(w/v)
5	PH	6.5

Table 2.2. Bromfield Medium Composition

S. No.	Constituents	Amount (g/L)
1	$(NH_4)_2SO_4$	0.25
2	KH_2PO_4	0.25
3	MgSO4	0.7
4	Carbon source	20
5	Yeast extract	1.0
6	PH	6.5

Table 2.3. Analysis of Iron Ore (XRF)

Composition	Weight Percent
Fe_2O_3	76.61
Al_2O_3	6.01
SiO_2	7.6
P_2O_5	0.9
TiO_2	0.2
Sum of Concentration	90.48

Figure 2.1 Locations of Iron ore mines of India (shown by the dots) collected from www.mapsofindia.com

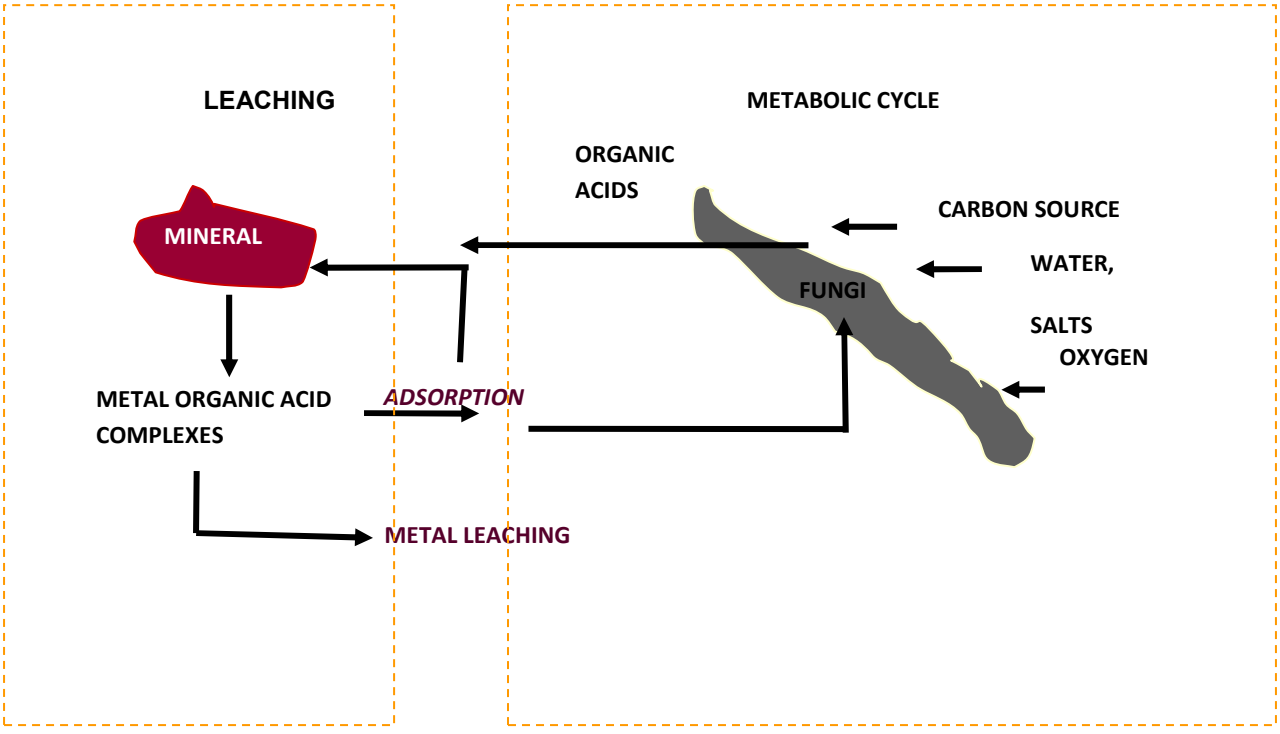

Figure 2.2 Mechanism of heterotrophic bioleaching

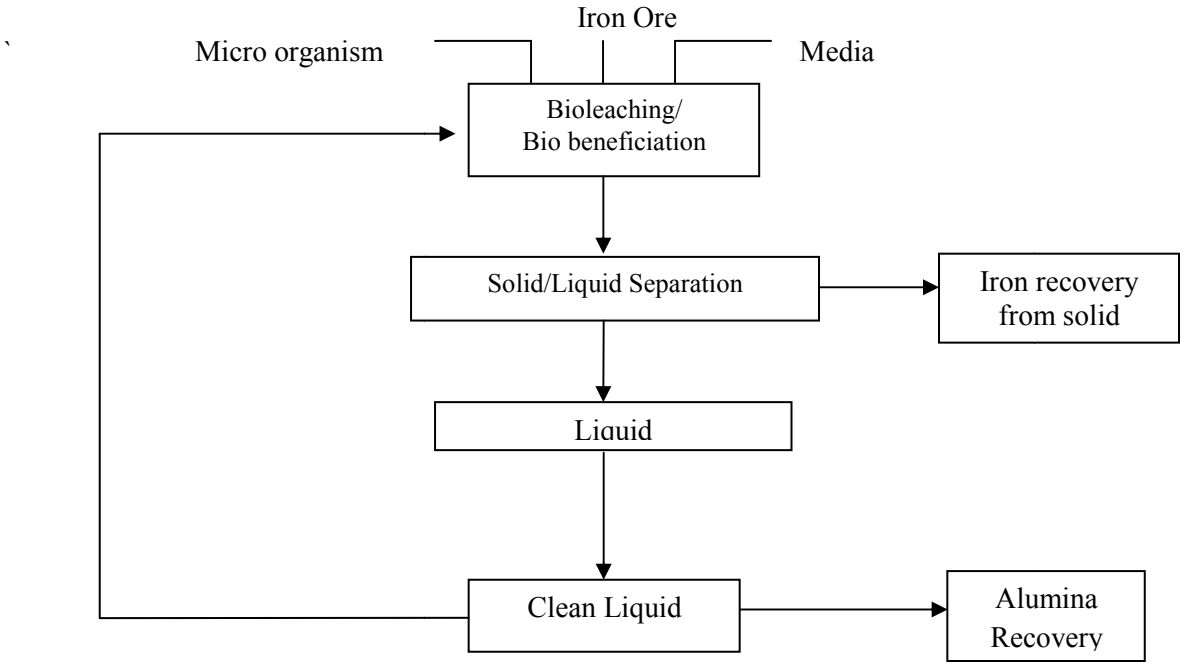

Figure 2.3 Tentative flow sheet for removal of Alumina from Iron ore

Figure2.4. ***Aspergillus flavus*** : **Colony morphology**

Figure2.5. ***Aspergillus fumigatus:*****Colony morphology**

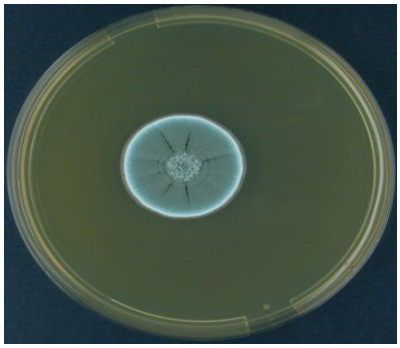

Figure 2 6. *Penicillium citrinum :* Colony morphology

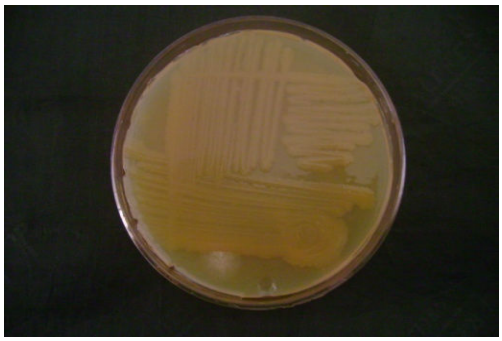

Figure 2.7. *Bacillus polymyxa :* Colony morphology

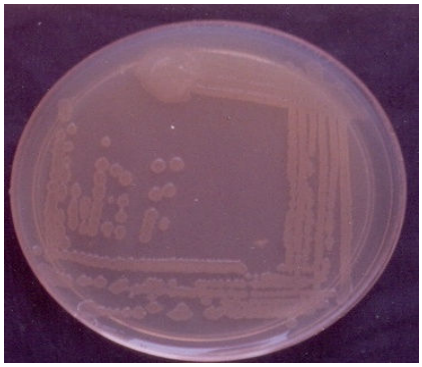

Figure 2.8. *Bacillus sphericus :* Colony morphology

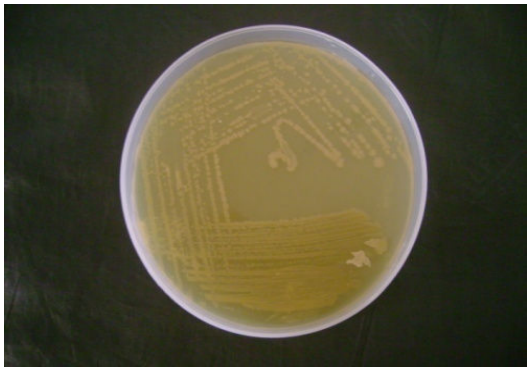

Figure 2.9. *Pseudomonas putida :* Colony morphology

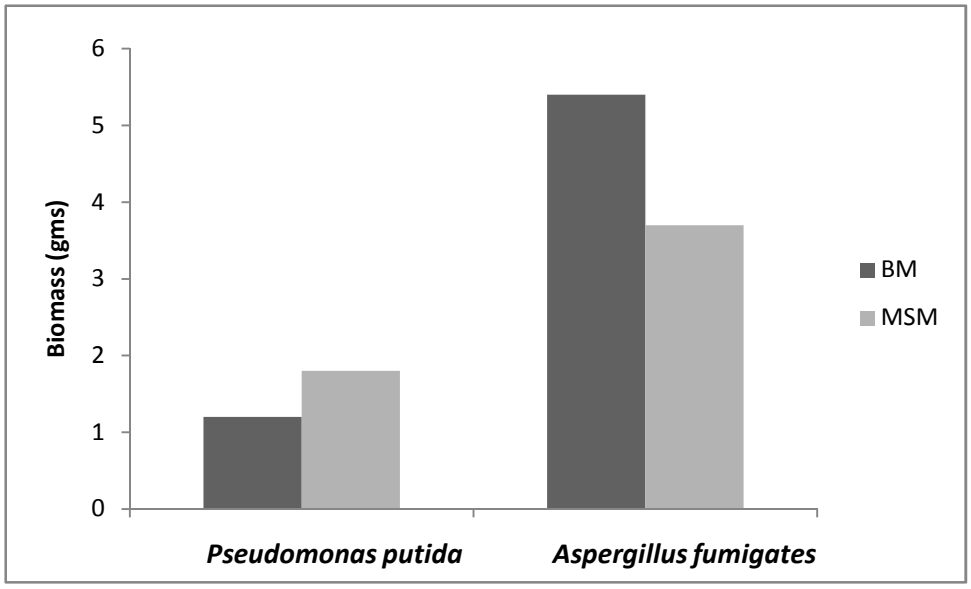

Figure 2.10. Effect of media on growth of *Pseudomonas putida* and *Aspergillus fumigates*.

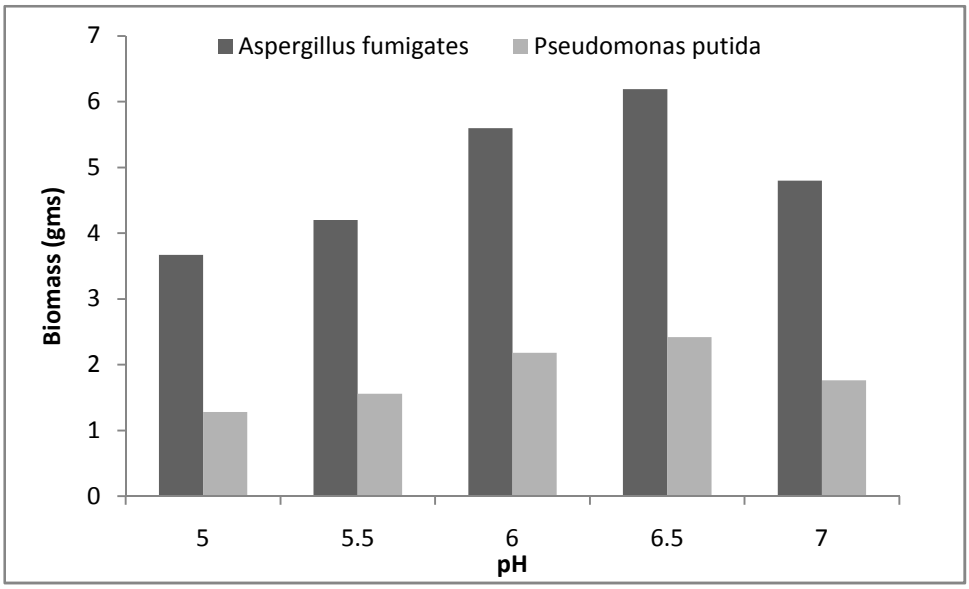

Figure 2.11. Effect of initial pH on growth of *Pseudomonas putida,* and *Aspergillus fumigates*.

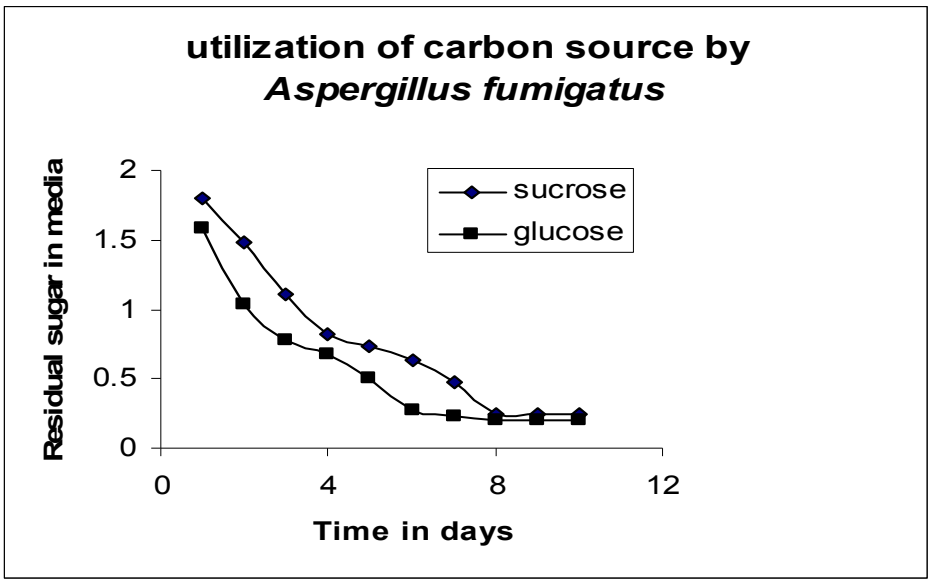

Figure 2.12 Utilization of carbon source by *Aspergillus fumigates*

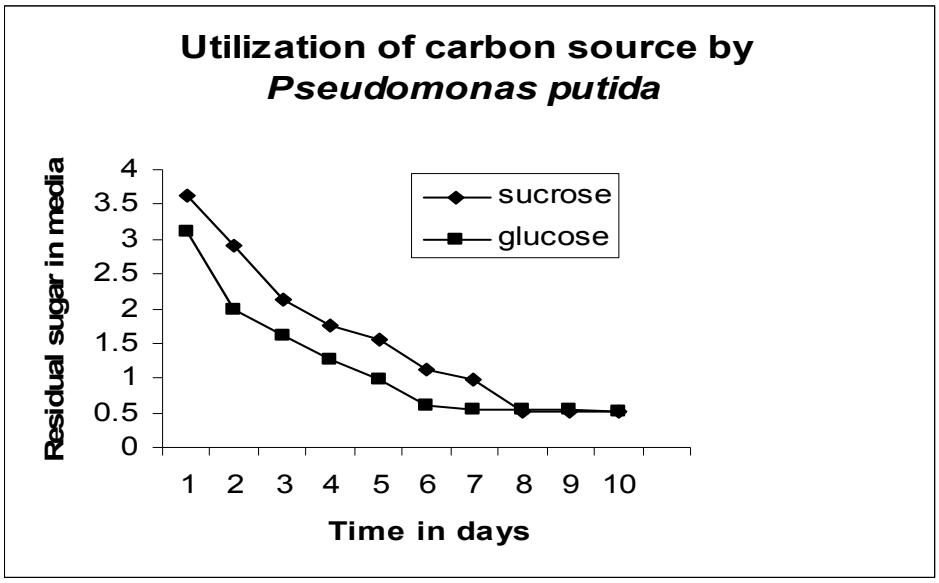

Figure 2.13 Utilization of carbon source by *Pseudomonas putida.*

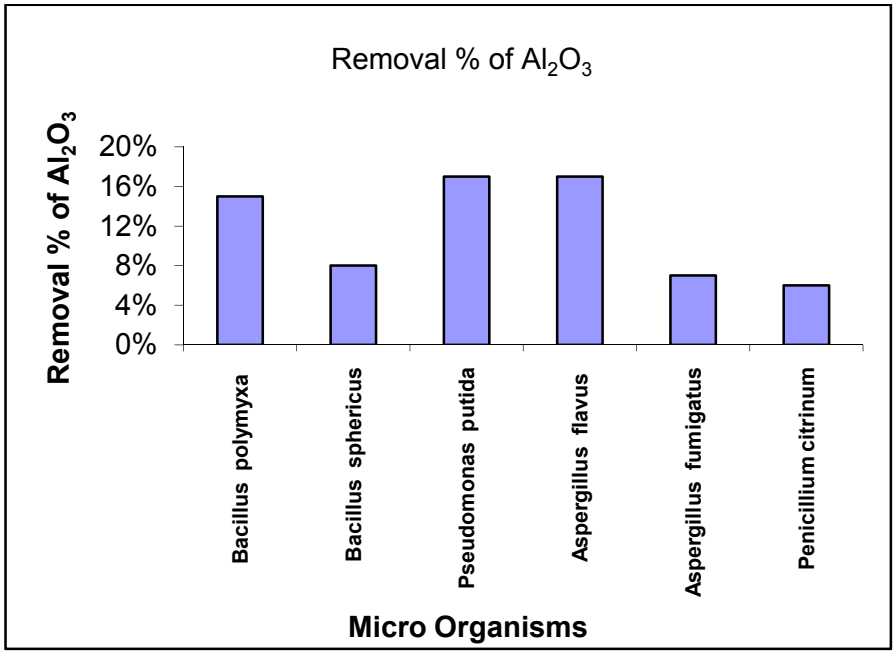

Figure 2.14 Removal percentage of Al_2O_3 using different fungal and bacterial strains.

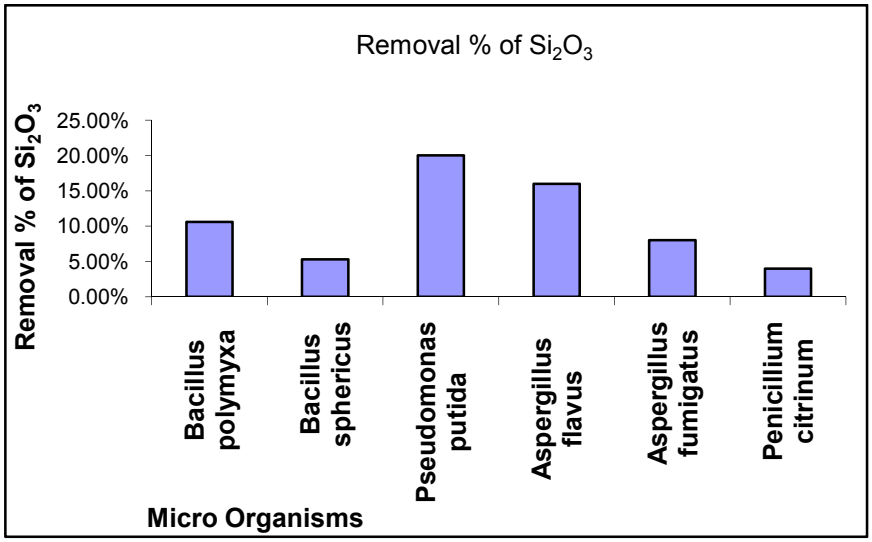

Figure 2.15 Removal percentages of Si_2O_3 using different fungal and bacterial strains.

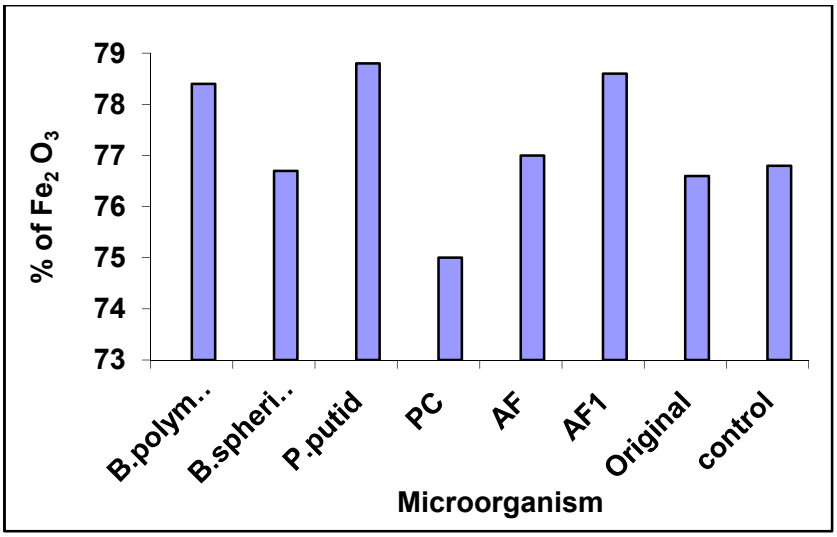

Figure 2.16 Initial Fe_2O_3 concentration of the Iron ore and final Fe_2O_3 concentration of the Iron ore after the action of different micro organisms (Determined by XRF). *Bacillus polymyxa, Bacillus sphericus, Pseudomonas putida,* **AF**- *Aspergillus fumigatus,* **PC**- *Penicillium citrinum,* AF1- *Aspergillus flavus*

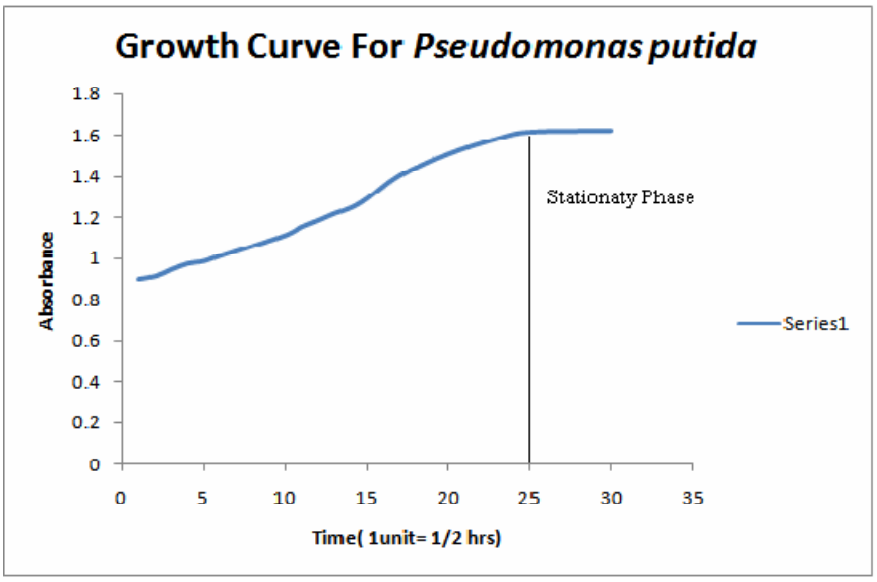

Figure 2.17 Growth curve for *Pseudomonas putida.*

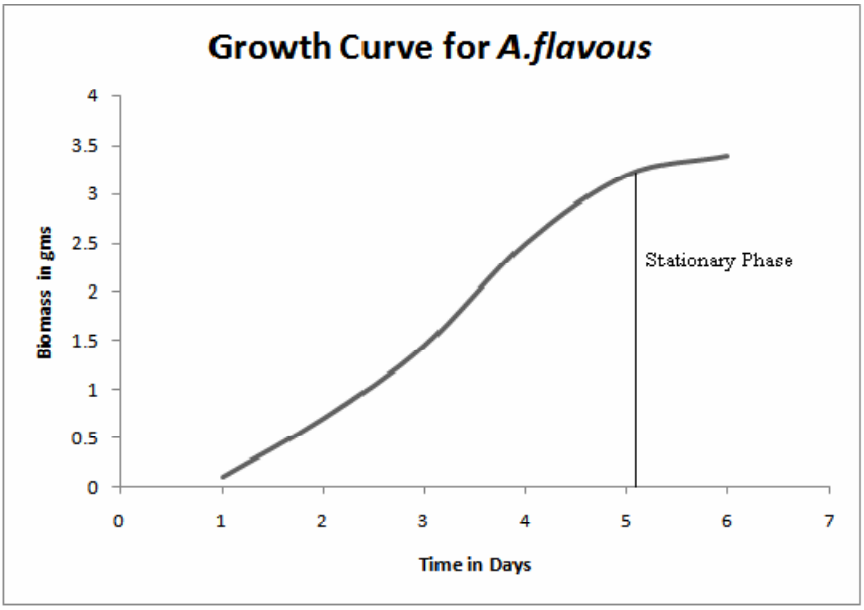

Figure 2.18 Growth curve for *Aspergillus flavus*

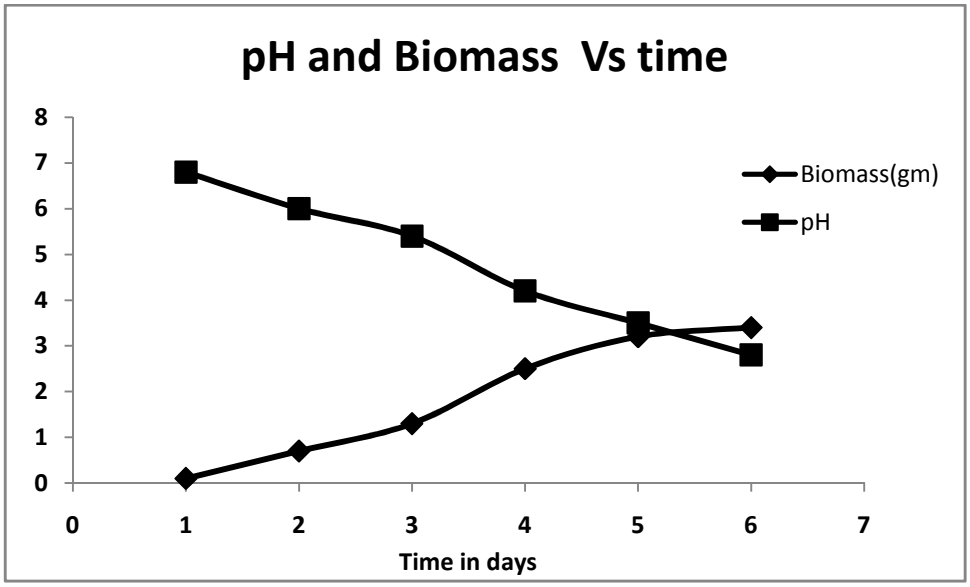

Figure 2.19 Decrease of the pH with biomass growth.

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Chapter 3

OPTIMIZATION OF PARAMETERS IN BIOLEACHING USING FACTORIAL DESIGN APPROACH: IN THE CONTEXT OF ZINC SULFIDE LEACHING USING *THIOBACILLUS FERROOXIDANS*.

3.1. INTRODUCTION

In the chapter three, the application of 2^4 level of full factorial design of experiments for zinc bioleaching using autotrophs manifested the screening of design parameters along with optimum parameter estimation. The purpose was to identify only the important variables that affect the response (desired goal) and their interactions (Montgomery, 2005; Box et *al.,* 1978). It also proposed the empirical model of the yield (zinc) as a function of major design parameters and the interactions among them using a statistical designing and analyzing software *MINITAB – 15*. Within the scope of this chapter, the batch investigation of Zinc sulphide ore (ZnS) using *Acidithiobacillus ferrooxidans* in aerobic condition was chosen as case study, keeping in view of the fact; no such studies had been ever conducted for microbial processing of zinc sulphide ore (ZnS). In the backdrop of the proposed objectivity, chapter 3 also explores the zinc metal

resources in India and abroad, their ores, leaching mechanism with autotrophs, the previous work done using *Acidithiobacillus ferrooxidans* apart from the development of statistical model relating yield and the major factors influencing the yield and quantization of the optimum factors.

Zinc, also known as spelter, is a metallic chemical element. It is the first element in group 12 of the periodic table. Zinc is, in some respects, chemically similar to magnesium, because its ion is of similar size and its only common oxidation state is +2. Zinc is the 24th most abundant element in the Earth's crust and has five stable isotopes. Centuries before zinc was recognized as a distinct element, zinc ores were used for making brass. Tubal-Cain, seven generations from Adam, is mentioned as being an "instructor in every artificer in brass and iron." An alloy containing 87 percent zinc has been found in prehistoric ruins in Transylvania. Metallic zinc was produced in the 13th century A.D., in India by reducing calamine with organic substances such as wool. The metal was rediscovered in Europe by Marggraf in 1746. He demonstrated that zinc could be obtained by reducing calamine with charcoal. Zinc is a bluish-white, lustrous metal. It is brittle at ordinary temperatures but malleable at 100 - 150^0C. It is a fair conductor of electricity, and burns in air at high red heat with evolution of white clouds of the oxide. It exhibits super plasticity. It has unusual electrical, thermal, optical, and solid-state properties that have not been fully investigated.

In terms of usage zinc is one of three most important metals in metals other than iron, coming after aluminum and copper (Anonim, 2001). In addition, zinc and its compounds have industrial and economic importance since they are widely used in many fields which range from paints, cosmetics, food, pharmaceutical, detergent, textile, leather, photography to storage batteries, electrical equipment or metallic coatings for corrosion protection, as well as an endless list of other capital applications (Gimenez-Romero et *al.,* 2004; Lang and Horanyi, 2005; Milsom and Meers, 1985). Zinc is also used extensively to galvanize other metals such as iron to prevent corrosion. The metal is employed to form numerous alloys with other metals. Brass, nickel silver, typewriter metal, commercial bronze, spring bronze, German silver, soft solder, and aluminum solder are some of the more important alloys. Large quantities of zinc are used to produce die castings, which are used extensively by the automotive, electrical, and hardware industries. An alloy of zinc called Prestal (R), consisting of 78 % zinc and 22 % aluminum, is

reported to be almost as strong as steel and as easy to mold as plastic. The alloy said to be so moldable that it can be molded into form using inexpensive ceramics or cement die casts.

Zinc oxide is a unique and very useful material for modern civilization. It is widely used in the manufacture of paints, rubber products, cosmetics, pharmaceuticals, floor coverings, plastics, printing inks, soap, storage batteries, textiles, electrical equipment, and other products. Lithopone, a mixture of zinc sulfide and barium sulfate, is an important pigment. Zinc sulfide is used in making luminous dials, X-ray and TV screens, and fluorescent lights.

The zinc chloride and chromate are also important compounds. Zinc is an essential element in the growth of human beings and animals. Tests show that zinc-deficient animals require 50 percent more food to gain the same weight as an animal supplied with sufficient zinc.

Moreover, zinc and its compounds play a role in various functions in both humans and animals. Deficiency of zinc leads to growth disturbance, taste impairment, anorexia, dermatitis, increasing sensitivity against various pathogens, as well as fetal and pregnancy complications. Zinc and its compounds have been used to restore these impaired functions (Chang et *al.,* 2006; Ueda et *al.,* 2006).

Zinc and its compounds are found in various forms, such as $ZnCO_3$, ZnS, etc., around the world. A little quantity of zinc is also present in almost all of the volcanic rocks. It is estimated that it forms 0.13% of the earth crust (Kirk et *al.*, 1952). The production of zinc in India is about 0.9 million tons per year (Jena, 2009). The principal ores of zinc are sphalerite (sulfide), smithsonite (carbonate), calamine (silicate), and franklinite (zinc, manganese, iron oxide).

3.1.1. Ores and Extraction

The most exploited zinc ore is sphalerite, a zinc sulfide. The largest exploitable deposits are found in Australia, Canada, and the United States. The conventional Zinc production includes froth flotation of the ore, roasting, and final extraction using electricity (electro winning). One method of zinc extraction involves roasting its ores to form the oxide and reducing the oxide with coal or carbon, with subsequent distillation of the metal. Zinc and zinc compounds are also products obtained from ores present in the nature as a result of leaching process applied by using

inorganic or organic reagents with hydrometallurgical methods. Even though sulphurous ores are among fundamental sources from which zinc is obtained, some other ores have become attractive since sulphurous ores are used up and they create sulfur emissions while they are processed (Abdel-Aal, 2000). Some oxidized ores, such as smithsonite ($ZnCO_3$), willemiyte (Zn_2SiO_4), hydrozincite ($2ZnCO_3.3Zn\ (OH)_2$), and zincite (ZnO) a hemimorphite ($Zn_2SiO_3.H_2O$) are other important ores containing zinc and alternatives for sulphurous ores (Zhao and Stanforth , 2000).

Because of the depletion of zinc concentrate ores of the sphalerite (ZnS) type, an important fraction of the world's zinc production currently derives from the processing of complex sulphide ores. The grade of the zinc in bulk sulphides concentrates is usually low (less than 10%), but this is enhanced by selective flotation which provides a Zn concentrate of metallurgical interest that is mixed with higher-grade concentrates. The process continues with the pyrometallurgical treatment. However, this is costly from the energy aspect and also suffers from another two drawbacks: (1). the release of part of the SO_2 into the atmosphere, from both the ore and the fuel used in the process; (2) the formation of ferrite as a result of the roasting temperature; this not only decreases the amount of zinc that can be recovered but also requires the use of concentrated sulphuric acid for dissolution. Alternative technologies, such as O_2–H_2SO_4 leaching (Veltmann et *al.*, 1980; Veltmann and Weir, 1982; Au-Yeung et *al.*, 1986), have been in use for some time but are not widespread. Methods based on direct leaching of the concentrate under hydrothermal conditions have been developed but they require high temperatures and render the zinc-dissolving process less selective (Demarthe and Georgeaux, 1978; Nogueira *et al.,* 1985; Ricketts *et al.,* 1989). Lower temperatures are required in chloride environments but the use of chlorides entails the adoption of costly anti-corrosion and anti-pollution stratagems even at relatively low temperatures (60–70^{0}C) (Mizoguchi and Habashi, 1981; Flett *et al.,* 1983; Buttinelli *et al.,* 1990). Used as an oxidizing and leaching agent, ferric sulphate is selective for sphalerite over other sulphides which may be present, creates fewer environmental problems and is economical; moreover, the regeneration of ferric ions to ferrous ones can be achieved easily by means of electrolysis or bacterial oxidation (Lacey and Lawson, 1970; Olem and Unz, 1980).

3.1.2. Microbial Extraction of Zinc

Microbes have geologically been active in mineral formation, mineral dissolution and sedimentation via direct action of their enzymes or indirectly through chemical action of their metabolic products. They act as agent of concentration dispersion or fractionation of mineral matter (Sharma et *al.,* 1991; Murr et *al.,* 1978; Scheiner et *al.,* 1989). Biohydrometallurgy is a rapidly evolving science dealing with extraction of metals like copper, zinc, silver, gold, uranium, selenium, nickel and many others from sulphide ore, oxide ores through solubilizing and metal accumulating properties of micro organisms.

Various micro organisms pose different trails to bring about metal extraction. *Acidithiobacillus ferrooxidans*, an autotrophic bacterium has received much attention because of its technological potential over the past few decades. The role of *Acidithiobacillus ferrooxidans* in bioleaching of metal sulphides is to catalyze the oxidation of metal sulphides through a number of enzymatic reactions, which include *rustycyanin, Fe (II)-cytochrome C-522 oxidoreductase*, *cytochrome C-522* and *cytochrome a*. Two mechanisms are suggested for the bioleaching of metal sulphides. They are:

(i) Direct mechanism which requires physical contact between bacteria and sulphide mineral surface.

$$ZnS + 0.5O_2 + 2H^+ \rightarrow Zn^{2+} + H_2O \quad (3.1)$$

(ii) Indirect mechanism in which the bacterially generated Fe (III) acts as a lixiviant for the solubilization of metals from their ores.

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (3.2)$$

$$2Fe^{3+} + ZnS \rightarrow 2Fe^{2+} + Zn^{2+} + S^0 \quad (3.3)$$

$$S^0 + 1.5O_2 + H_2O \rightarrow H_2SO_4 \quad (3.4)$$

In practice, however, both of the mechanisms may operate simultaneously in a complementary manner (Sharma et *al.,* 1991; Murr et *al.,* 1978; Scheiner et *al.,* 1989; Norris et *al.,* 1988; Volesky, 1990).

Within the scope of this chapter, the present batch investigations have been undertaken to develop an effective microbial leaching process of Zinc sulphide ore (ZnS) using

Acidithiobacillus ferrooxidans in aerobic condition. Attempts have been made to optimize process parameters like initial zinc sulphide loading (pulp density), pH, temperature and shaking speed to leach out maximum quantity of Zinc using suspended batch culture of bacteria.

3.2. REVIEW OF LITERATURE

The leaching of a sphaleritic flotation concentrate in an acidic ferric sulphate solution is described by an electrochemical charge-transfer model by Verban et *al.,* 1986., have demonstrated the bioleaching of Zinc sulfide ore using *Acidithiobacillus ferrooxidans* and tried to optimize the parameters individually. Hursit et *al.,* 2009, have investigated the dissolution kinetics of smithsonite ore in aqueous gluconic acid solutions in a batch reactor by using the parameters such as temperature, acid concentration, particle size and stirring speed.

Even though the reaction mechanism has been thoroughly investigated (Verban and Crundwell, 1986; Crundwell, 1987), and it has been established that the dissolution kinetics are connected with the iron content of the mineral concerned (Palencia Perez and Dutrizac, 1990), the effects of experimental factors and of their interactions on the results of the process are still not perfectly understood. In previous investigations in this field the 'one-factor-at-a-time' method had been used, in which one factor varies up to the value maximizing the value of the dependent variable, while the levels of the other factors are kept constant. This method fails whenever the maximizing value of the varying factor is not independent of the levels of the other factors, i.e. interactions may exist among the variables. These interactions, if present, are easily detected when a factorial strategy is followed. In addition, factorial design permits more precision in estimation of the effects of the factors than the 'one-factor-at-a-time' design, at the same number of experimental runs. Moreover, the whole set of experimental tests permits to estimate an empirical model relating the yield with the parameters, expressed by polynomial equations, that comes in handy when the physics involved are unknown or too complicated to study and postulate a theoretical model (Box et *al.,* 1978). The empirical model permits the derivation of a response surface which provides zinc recovery levels in relation to the levels of the factors adopted in the experiments.

Many researchers have followed the statistical designing of experiments for optimizing the leaching processes of different metals (Sayan et *al.,* 2004; Sahoo et *al.,* 2001; Lozano Blanco et *al.,* 1999).

P. Massacci. et *al.,* 1998, ascertained the influence of the main factors involved in the leaching of sphalerite with ferric sulphate and to construct a statistical model which can predict levels of zinc extraction as a function of those of the main factors affecting the leaching. The factors taken into consideration were temperature, oxidant concentration, particle-size distribution and solid/liquid ratio.

3.3. FACTORIAL DESIGN

In any process, there may be a large number of input variables (factors) that may be assumed *a priori* to affect the process. Screening experiments are conducted to determine the inputs and interactions of inputs that influence the process significantly. In general the relationship between the inputs and outputs can be represented as

$$y = f(x_1, x_2, x_3 \dots, x_p) + e \tag{3.5}$$

where x_i, i=1-p are the factors, 'e' is the random and systematic error and y is the response variable. Approximating this equation by using Taylor series expansion:

$$y = b_0 + b_1x_1 + b_2x_2 + \cdots + b_px_p + \cdots + b_{ij}x_{ij} + \cdots + b_{jp}x_{jp} + \cdots + b_{11}x_1^2 + \cdots + b_{ii}x_i^2 + \cdots + b_{pp}x_p^2 + \cdots + Higher\ order\ terms + e \tag{3.6}$$

a polynomial response surface model is obtained where b_i denotes the parameters of the model. The first task is to determine the factors (x_i) and the interactions (x_ix_j, $x_ix_jx_k$ and higher order interactions) that influence y. Then, the coefficients like b_i, b_{ij}, b_{ijk} of the influential inputs and interactions are computed. These parameters of the response surface models can be determined by least squares fitting of the model to experimental data.

In statistics, a full factorial experiment is an experiment whose design consists of two or more factors, each with discrete possible values or "levels", and whose experimental units take on all possible combinations of these levels across all such factors. A full factorial design may

also be called a fully-crossed design. Such an experiment allows studying the effect of each factor on the response variable, as well as the effects of interactions between factors on the response variable.

For the vast majority of factorial experiments, each factor has only two levels. For example, with two factors each taking two levels, a factorial experiment would have four treatment combinations in total, and is usually called a *2×2 factorial design*.

Factorial designs were used in the 19th century by John Bennet Lawes and Joseph Henry Gilbert of the Rothamsted Experimental Station (Yates et *al.,* 1963). Factorial designs were used in the 19th century by John Bennet Lawes and Joseph Henry Gilbert of the Rothamsted Experimental Station (Yates et *al.,* 1963). Ronald Fisher argued in 1926 that "complex" designs (such as factorial designs) were more efficient than studying one factor at a time (Fisher, 1926). Fisher wrote, "No aphorism is more frequently repeated in connection with field trials, than that we must ask Nature few questions, or, ideally, one question, at a time. The writer is convinced that this view is wholly mistaken. Nature, he suggests, will best respond to a logical and carefully thought out questionnaire". A factorial design allows the effect of several factors and even interactions between them to be determined with the same number of trials as are necessary to determine any one of the effects by itself with the same degree of accuracy. Frank Yates made significant contributions, particularly in the analysis of designs, by the *Yates Analysis*. The term "factorial" may not have been used in print before 1935, when Fisher used it in his book *The Design of Experiments* (Yates et *al.,* 1963).

Designs can involve many independent variables. As an example, the effects of three input variables can be evaluated in eight experimental conditions shown in fig. 3.1 To save space, the points in a two-level factorial experiment are often abbreviated with strings of plus and minus signs. The strings have as many symbols as factors, and their values dictate the level of each factor: conventionally, − for the first (or low) level, and + for the second (or high) level. The points in a 2^2 factorial experiment can thus be represented as − −, + −, − +, and + +.

A factorial experiment can be analyzed using ANOVA (Analysis of Variance) or regression analysis. It is relatively easy to estimate the main effect for a factor. To compute the

main effect of a factor "A", subtract the average response of all experimental runs for which A was at its low (or first) level from the average response of all experimental runs for which A was at its high (or second) level. Other useful exploratory analysis tools for factorial experiments include main effects plots, interaction plots, and a normal probability plot of the estimated effects. When the factors are continuous, two-level factorial designs assume that the effects are linear. If a quadratic effect is expected for a factor, a more complicated experiment should be used, such as a central composite design. Optimization of factors that could have quadratic effects on the response is the primary goal of response surface methodology.

3.4. MATERIALS AND METHODS

3.4.1. Collection of Micro Organism and Growth

An acidophilic mesophile iron oxidizer *Acidithiobacillus ferrooxidans* (MTCC-2361) used in the present study which was obtained from the Institute of Microbial Technology, CSIR, Chandigarh. The prescribed medium is shown in Table 3.1. pH has been adjusted with 0.1 N H_2SO_4. The inoculum was added and incubated at a constant temperature of 30^0C with a constant shaking speed at 60 rpm. The stock and pre-inoculum cultures were maintained in the same medium under similar conditions. The stock cultures were subcultured every two weeks.

3.4.2. Collection of Zinc Sulphide Ore and Analysis

The Zinc sulphide ore (ZnS) was collected from Central Electrochemical Research Institute of CSIR, Karaikudi. The sample was first crushed in a jaw crusher and then sewed by using a mesh to get the required particles size (-200 to +240). The ore was analyzed for Zn (II) and SiO_2 contents and found to be 63.25% (w/w) and 18.56% (w/w) respectively.

3.4.3. Design of Experiments (DOE)

In this work, statistical design of experimental methods (DOE) was applied to study the leachability of Zinc sulphide. This is a simultaneous study of determining several major process variables influencing the yield (Barrentine, 1999; Montgomery, 2005). Several factors were changed in a systematic way so as to ensure the reliable and independent study of the main

factors and interactions among them, which are sensitive to the process. The purpose was to identify only the important variables that affect the response and their interactions (Montgomery, 2005; Box et *al.,* 1978). The significance of each factor and their interactive effects were evaluated using statistical design of experiments two-level full factorial designs 2^4. The major influencing factors were chosen to be 4 numbers; hence, 2^4 or 16 numbers of experiments were resulted combining the high and low level values of each variable. There were additional 6 numbers of experiments were performed repetitively at base level. Base level means the combination of the intermediate values (mean of high and low level values) of each variable. The major objective was to maximize the solubilization of Zinc sulphide (response) using *Acidithiobacillus ferrooxidans*.

3.4.4. Variables

This study determines the influence of some of the factors in the bacterial leaching of Zinc sulphide ore using *Acidithiobacillus ferrooxidans* and quantifies them to ensure that the influence is getting transformed into measurable response. The potential design factors were classified as controlled-factors and held-constant factors. The controlled factors (Table 3.2) were the factors selected for the present study. The held constant factors, like inoculum size, leaching time and particle size were held at a specific level for the present study though their effect on the response cannot be completely overruled.

3.4.5. Statistical Analysis

All the experimental designs and results were statistically analyzed by using a statistical designing and analyzing software 'MINITAB – 15'.

3.4.6. Normal Probability Plots of Effects

When analyzing data from factorials, occasionally high order interactions occur and as such normal probability plots are used (Daniel, 1959) to estimate the significant factors. This is the plot of the actual value of the estimated effects against their cumulative normal probabilities (Daniel, 1959).The nominal effects are normally distributed with mean zero and variance (σ^2) and will tend to fall along a straight line, whereas significant effects will have nonzero means

and will not lie along the straight line. Effects in the statistical designs are done by averaging the responses that are applicable to the level of each factor. The difference between the average responses at the two levels of each factor is an indication of the significance of that factor in influencing the response measured. Expressed mathematically, the single effects caused by the variation of the input parameters are calculated with the following formula:

$$Effect = \frac{2}{m}\sum_{i=1}^{m}(algebraic\ sign\ of\ constant \times R_{\text{i,observed}})$$

$$Where$$

$$m \rightarrow number\ of\ runs$$

$$R \rightarrow Responce$$

3.4.7. Pareto Plots of Effects

A Pareto chart is a special type of bar chart where the values being plotted are arranged in descending order. As we are studying the effects of different factors on the response, it will give us the descending order of effects exerted by different variables and interactions between them. It helps us to set the priority levels while designing the process. These Pareto charts were prepared based on 80-20 rule, which states that 80 percent of effects come from 20 percent of the various causes (Koch, 2001).

3.4.8. Graphical Residual Analysis

The normal plotting of residuals provides a diagnostic test for any tentatively entertained model (Montgomery, 2005; Box et *al.*, 1978). The normal probability plots of the residuals for the data tests the hypothesis that the residuals have a normal distribution. This should be a straight line if the residuals have a normal distribution. A plot of residuals versus fits (fitted model values) tests the assumption or hypothesis that the variations are the same in each combination.

3.4.9. Tests for Curvature Using Center Points

Adding centre points to the design provide protection against curvature from second order effects as well as allowing an independent estimation of error (Montgomery, 2005). If $\bar{Y}_C$ is the average percentage leaching of Zinc from N_C runs at the centre and $\bar{Y}_F$ the average percentage leaching of Zinc from the runs at the factorial points under study and the difference ($\bar{Y}_F - \bar{Y}_C$) is small, then the centre points lie on or near the plane passing through the factorial points and hence there is no quadratic curve. On the other hand, if $\bar{Y}_F - \bar{Y}_C$ is large, then quadratic curvature is present.

3.4.10. Experimental Design and Sampling

All the leaching experiments were carried out in 250ml conical flasks with 100ml leaching solution in it. 20% (v/v) inoculum was added to them. Different pHs were maintained by using pH meter and 0.1N H_2SO_4. Different temperatures and shaking speeds were maintained by incubating the flasks in different shaker incubators for 8 days.

3.4.11. Determination of Zn (II) Concentration

At the end of leaching 50ml of the leached solution was taken out of the flasks and centrifuged at 100 rpm for 10 min in Remi R- 8C laboratory centrifuge at room temperature to remove biomass and un-solubilized ore materials. The clear solution was digested with 1:1 H_2SO_4 and after digestion the solution was diluted with de-mineralized water and filtered for separating silica (Bandyopadhyay and Banik, 1995). The filtrate was made up to the mark in a 250ml volumetric flask. The amount of Zn (II) was determined by using Atomic Absorption Spectroscopy (AAS).

3.5. RESULTS AND DISCUSSION

3.5.1. Significant Factors

The variables and their levels of 2^4 full factorial designs are given in Table 3.2. The higher level of variable was designated as '+' and lower level was designated as '-'. The matrix

for four variables varied at two levels (+,-) and corresponding metal leaching percentage Y_{Zn} is shown in Table 3.3. According to the design of experiments principle, six experiments were carried out at base level (initial Zn loading, 27.5 Kg/m^3; pH, 3.0; temperature, 40^{0}C; shaking speed, 75 rpm) to estimate error and standard deviation.

The regression model equation with interaction terms can be written as:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4 + b_{123}x_1x_2x_3 + b_{124}x_1x_2x_4 + b_{234}x_2x_3x_4 + b_{1234}x_1x_2x_3x_4 \quad (3.7)$$

$Where$

$Y = The\ percentage\ of\ metal\ leaching$

$b = model\ coefficients$

x_1, x_2, x_3, x_4= dimensionless coded factors for initial Zn loading, pH, temperature, shaking speed respectively.

Neglecting the coefficients (Table 3.4) not significant at 95% confidence level (which will have a standardized effect beyond 2.16), the regression eq. (3.7) becomes:

$$Y = 60.533 - 1902x_1 - 6.2x_2 - 3.723x_3 - 1.695x_4 \quad (3.8)$$

The negative signs in some of the variables of the predictive model equation indicate that in order to maximize bioleaching of Zinc sulphide ores, these factors must be kept in low levels. The positive signs mean the factors must be kept in high levels.

According to the stratagem presented in Table 3.3, 16 number of experiments were conducted to estimate the percentage of zinc leached out (Y) using the requisite amount of *initial Zn loading, pH, temperature, and shaking speed* provided in Table 3.2 . Regression of eq. (3.7) after substituting 16 number of Y values (Table 3.3) and the values of initial *Zn loading, pH, temperature, and shaking speed* according to the stratagem presented for the dimensionless coded factors, the model coefficients were determined. The standardized effects of individual

factors as presented in Table 3.4 were the difference between the average response considering the higher and lower level values of the concerned influencing factors. The standardized effects of the interaction terms ($x_i x_j$) is the difference between the average effect of the first factor for one level of the second factor and that for the other level of the second factor. The significant parameters were chosen by the probability plot of standardized effects (fig. 3.2). When the effects of individual factors were assessed using the factorial design, A (initial Zn loading), B (pH), C (temperature) and D (shaking speed) showed that they were statistically significant factors since they have nonzero means. All the interactions were statistically insignificant because they do not differ much from normal distribution (zero mean). So there were no significant interactions between individual factors.

Fig. 3.3 shows the Pareto chart of standardized effects. It gives the factors which are having a standardized effect that is beyond 95% confidence level (beyond the line at 2.16). It shows that pH is the most significant factor in the Zinc sulphide bioleaching process. Also it shows the descending order of the effects of different factors and their interactions.

The six observed recoveries at the centre were 63.80%, 63.4%, 63.1%, 63.214%, 63.103% and 63.842% (Table 3.3). The average of these six centre points is 63.40983%. The average of the 16 runs for base design (Table 3.3) is 57.6716%. Since these two averages are not very similar (difference of 5.8%), it is suspected that there is a curvature present. The test for nonlinearity, however, does not tell which factor(s) contain the curvature except its existence (Barrentine, 1999). However, for the purpose of this study (screening of factors), it was assumed the linearity assumption holds here very approximately (Montgomery, 2005).

Fig. 3.4 is a normal plot of residuals. As can be seen, all residuals lie on a straight line with linear correlation coefficient of 0.979, which shows that the residuals were distributed normally. A plot of residuals versus predicted values tests the assumption or hypothesis that the variations are the same in each combination (fig. 3.4) i.e., the random errors are distributed with mean zero and constant variance. All residuals were distributed between −3 and +4. Since the residuals were distributed normally with constant variance, mean zero and independently (figs. 3.4 and 3.5), it can be concluded that eq. (3.8) was in excellent agreement with the experimental data. In other words, the underlying assumptions about the errors were satisfied.

3.5.2. Influence of Factors on Leaching

3.5.2.1. Main effects

The main effects of all the four individual factors used for the study were shown in fig. 3.6. It shows that higher leaching of Zinc sulphide ore is obtained at low pulp density of 25Kg/m^3. The reduction in the rate of bacterial leaching at higher pulp density can be attributed to ineffective homogeneous mixing of solids and liquids leading to gas transfer limitation (Ochoa et *al.,* 1999; Gericke et *al.,* 2001) because the liquid becomes too thick (high viscosity) for efficient gas transfer to the cells. The figure shows higher percentage leaching at low pH. The tolerance of acidophiles to most metals in low pH media probably results from effective competition by H^+ ions for negatively charged sites at the cell surface (Volesky, 1990). However, it is needless to mention that *Acidithiobacillus ferrooxidans* is a gram-negative, acidophilic, mesophilic and chemoautotrophic bacteria.

In bioleaching of Zn (II) from concentrates, pH sharply decreases with time which results in suppression of bacterial activity. In order to avoid this negative effect, lime and/or calcite is added to the leach suspension. Fig. 3.6 shows that percentage leaching is decreasing at higher temperature. The intensity of bacterial leaching for Zinc sulphide ore depends on the rate of supply and dissolution of O_2 and CO_2. The dissolution of O_2 and CO_2 decreases with the increase in temperature (Volesky, 1990). Fig. 3.6 also shows that the response is low at higher level of shaking speed. Normally the enzymes responsible for bioleaching of Zinc sulphide ore will be secreted more at low shaking speeds.

3.5.2.2. Interaction effects

The interaction effects were shown in fig. 3.7. From the normal plots of the interaction effects (fig. 3.7), it can be concluded that no interaction is statistically significant (If there is any interaction present, the two lines will intersect). From fig. 3.7, the lack of interaction has been evident between the factors except that between pH and temperature (those two lines are not parallel), even though that interaction is statistically insignificant.

3.6. CONCLUSION

The purpose of this diagnostic two-level design was to obtain experimental data which served as an initial approach to optimization of bacterial leaching of Zinc sulphide ore using *Acidithiobacillus ferrooxidans*. It can also be utilized in establishing the factors with significant effects on the response and whether these effects are positive or negative. As a result of this diagnostic experiment, all the parameters considered in the present study including shaking speed were found to be statistically significant operating parameters. In the ranges under consideration, the interaction between the variables was observed to be statistically insignificant. The results also showed that recovery was maximized at low pH and low pulp density.

The difference in average response of the centre points compared to that of the factorial points indicated that there was a possibility of a curvature, hence, a new experimental design at three levels will have to be carried out to estimate not only the linear effects but also quadratic effects for maximal zinc recovery in the region defined by this diagnostic experiments. A further investigation with a response surface model may corroborate the estimated optimum influencing factors for zinc sulfide leaching using *Acidithiobacillus ferrooxidans*.

Table 3.1 Composition of the prescribed media for *Acidithiobacillus ferrooxidans*

Component	Amount
KH_2PO_4	0.4 gm/L
$MgSO_4.7H_2O$	0.4 gm/L
$(NH_4)_2SO_2$	0.4 gm/L
$FeSO_4$	33.3 gm/L
pH	1.4

Table 3.2 The controlled factors of 2^4 Factorial design for Zn leaching

Leaching time: 192 hrs; Particle size: 135 µm; Inoculum size: 20 % (v/v)

Variable	Low level	Base level	High level
Initial Zn loading	25 Kg/m^3	27.5 Kg/m^3	30 Kg/m^3
pH	2.5	3	3.5
Temperature	35^0C	40^0C	45^0C
Shaking speed	60 rpm	75 rpm	90 rpm

Table 3.3 2^4 Full factorial design for Zinc leaching

'-': Low level, '+': High level, x_1: initial Zn loading, x_2: pH, x_3: temperature, x_4: shaking speed

Observation	Coded factors				Extraction (%)
	X_1	X_2	X_3	X_4	
1	-	-	-	-	72.6
2	+	-	-	-	68
3	-	+	-	-	58.4
4	+	+	-	-	54.3
5	-	-	+	-	63.888
6	+	-	+	-	59.84
7	-	+	+	-	51.392
8	+	+	+	-	47.784
9	-	-	-	+	68.679
10	+	-	-	+	64.328
11	-	+	-	+	55.246
12	+	+	-	+	51.386
13	-	-	+	+	59.967
14	+	-	+	+	56.168
15	-	+	+	+	48.238
16	+	+	+	+	44.825
17	0	0	0	0	63.8
18	0	0	0	0	63.4
19	0	0	0	0	63.1
20	0	0	0	0	63.214
21	0	0	0	0	63.103
22	0	0	0	0	63.842

Table 3.4 Model coefficients (eq. 3.8) and Standardized effects of variables.

Code	Coefficient	Standardized Effect
Average	60.533	
x_1	-1.902	-5.19
x_2	-6.2	-16.72
x_3	-3.723	-9.99
x_4	-1.695	-4.51
x_1x_2	0.114	0.30
x_1x_3	0.128	0.34
x_1x_4	0.058	0.16
x_2x_3	0.416	1.11
x_2x_4	0.188	0.50
x_3x_4	-0.003	-0.01
$x_1x_2x_3$	-0.01	-0.03
$x_1x_2x_4$	-0.004	-0.01
$x_1x_3x_4$	-0.003	-0.01
$x_2x_3x_4$	-0.003	-0.01
$x_1x_2x_3x_4$	-0.003	-0.01

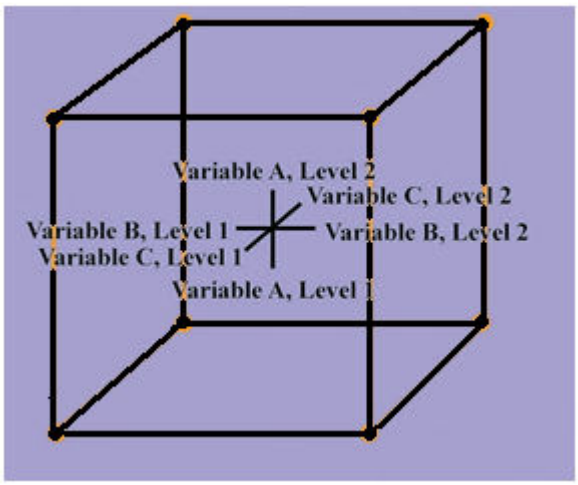

Figure 3.1 **2^3 factorial design. Three independent variables, two levels of each variable and eight test conditions.**

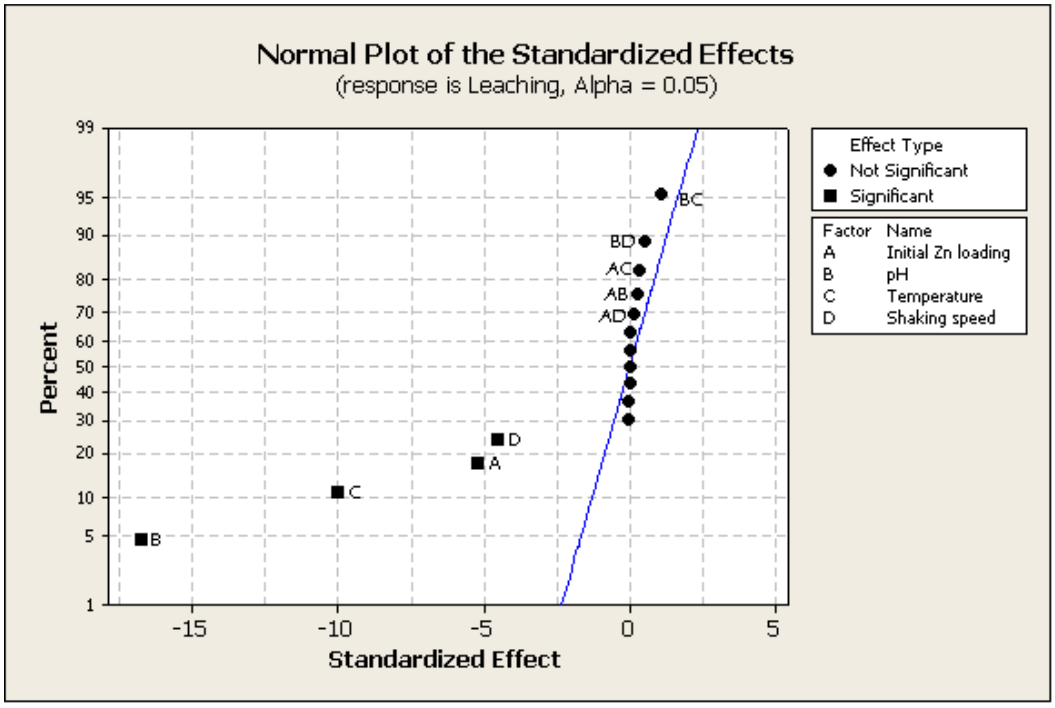

Figure 3.2 **Normal plot of effects of factors and their interactions. A, B, C, D are factors; AB, AC, AD, AE, BC, BD, BE, CE, CE are interaction among the factors. Generated by MINITAB 15.**

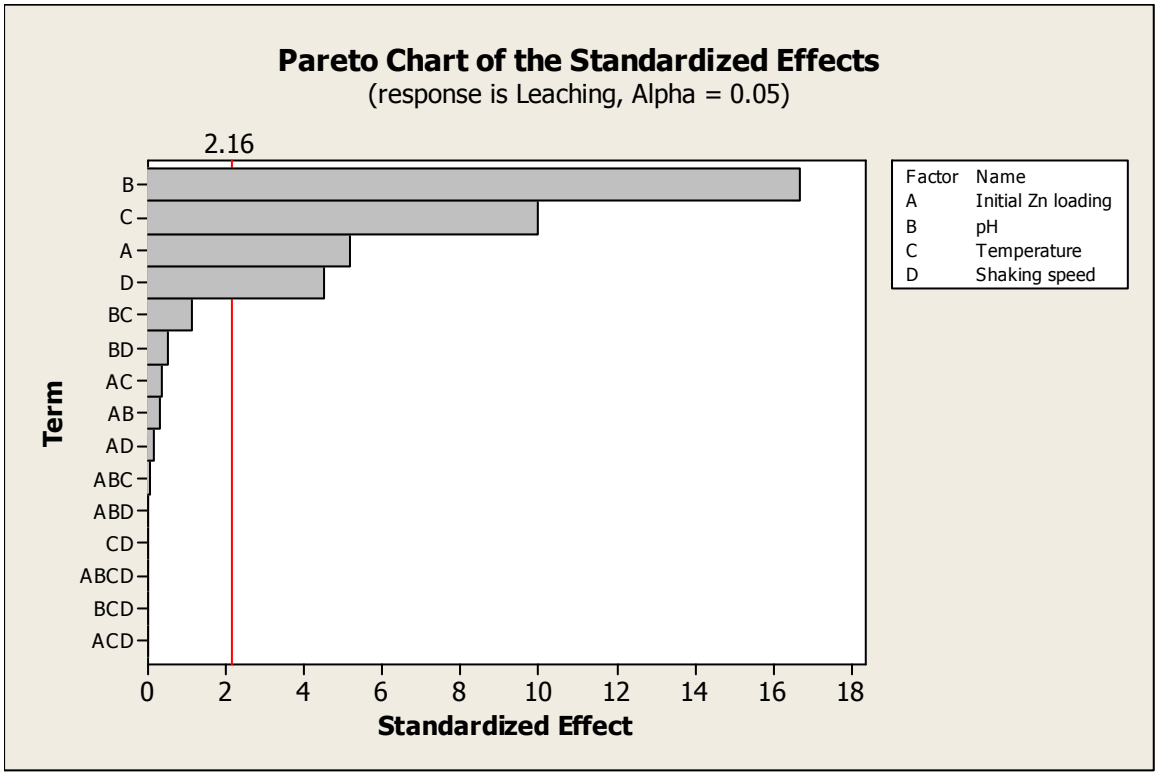

Figure 3.3 Pareto chart of standardized effects. Generated by MINITAB 15.

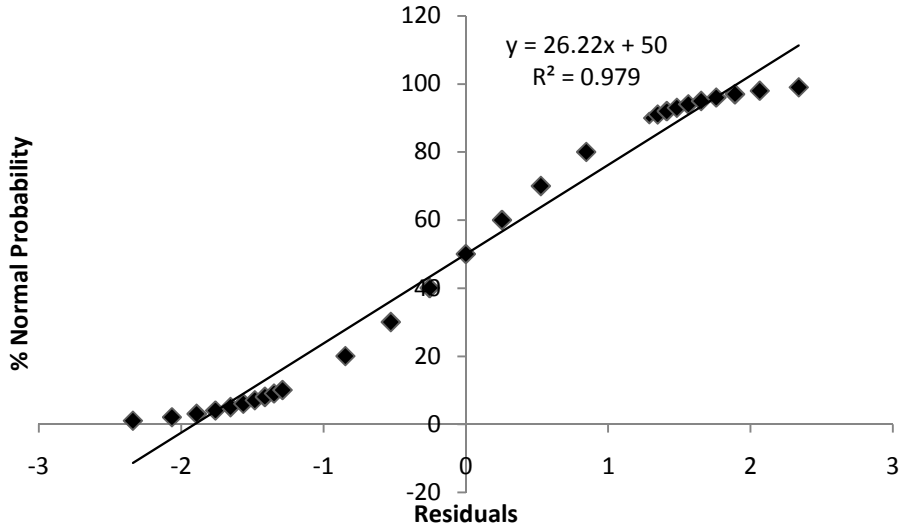

Figure 3.4 Normal plot of residuals.

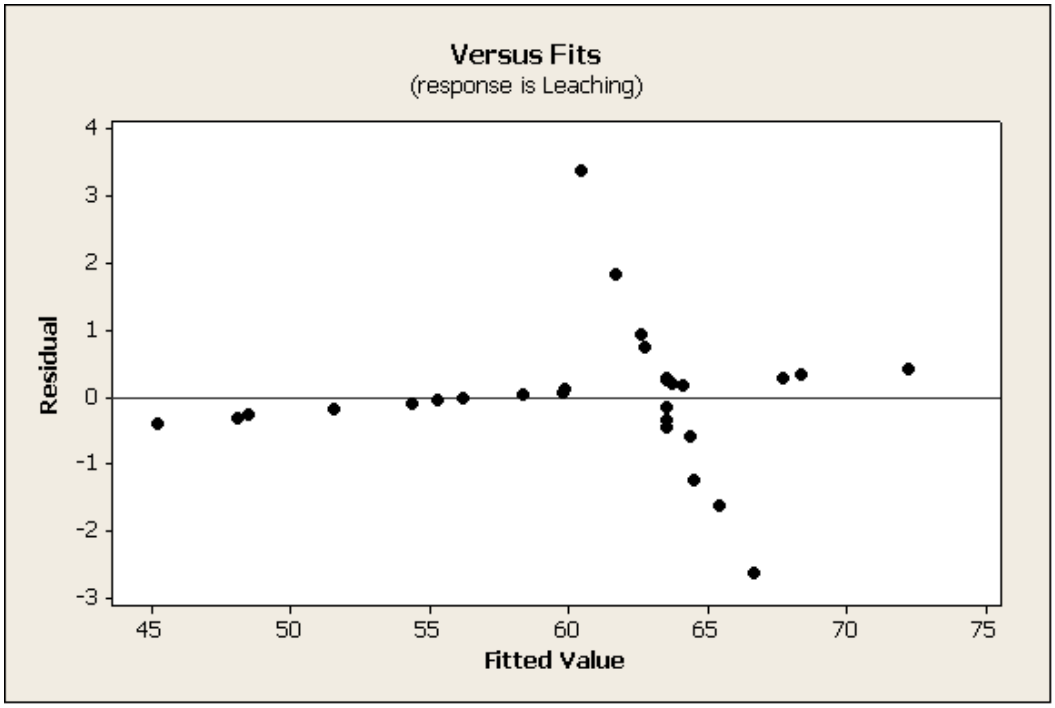

Figure 3.5 Plot of residuals versus predicted recoveries. Generated by MINITAB 15.

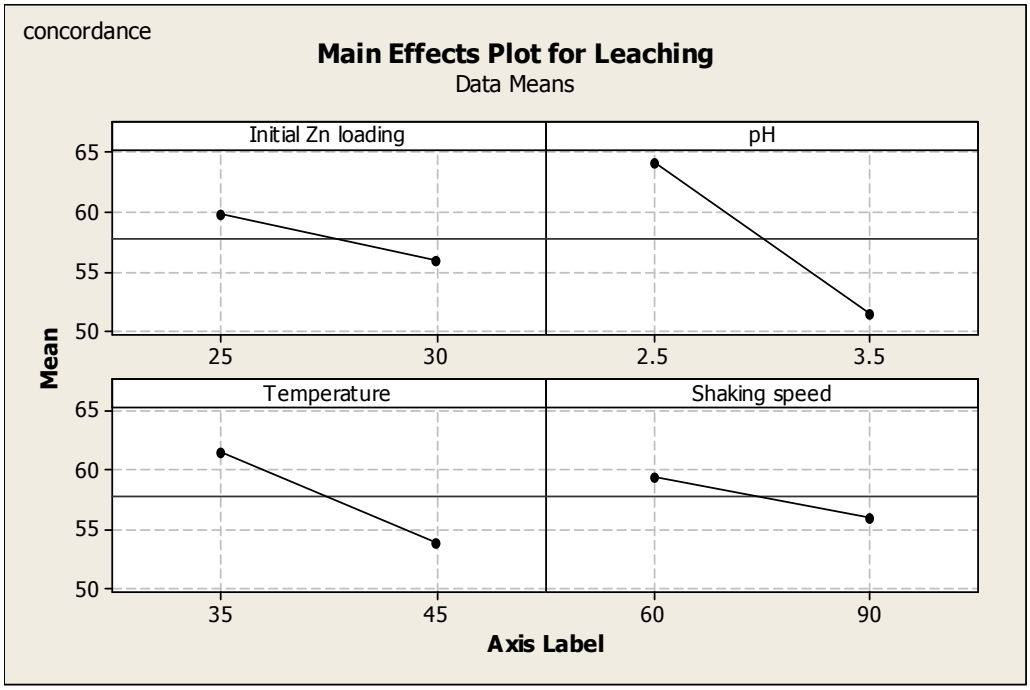

Figure 3.6 Main effects plot generated by using MINITAB 15

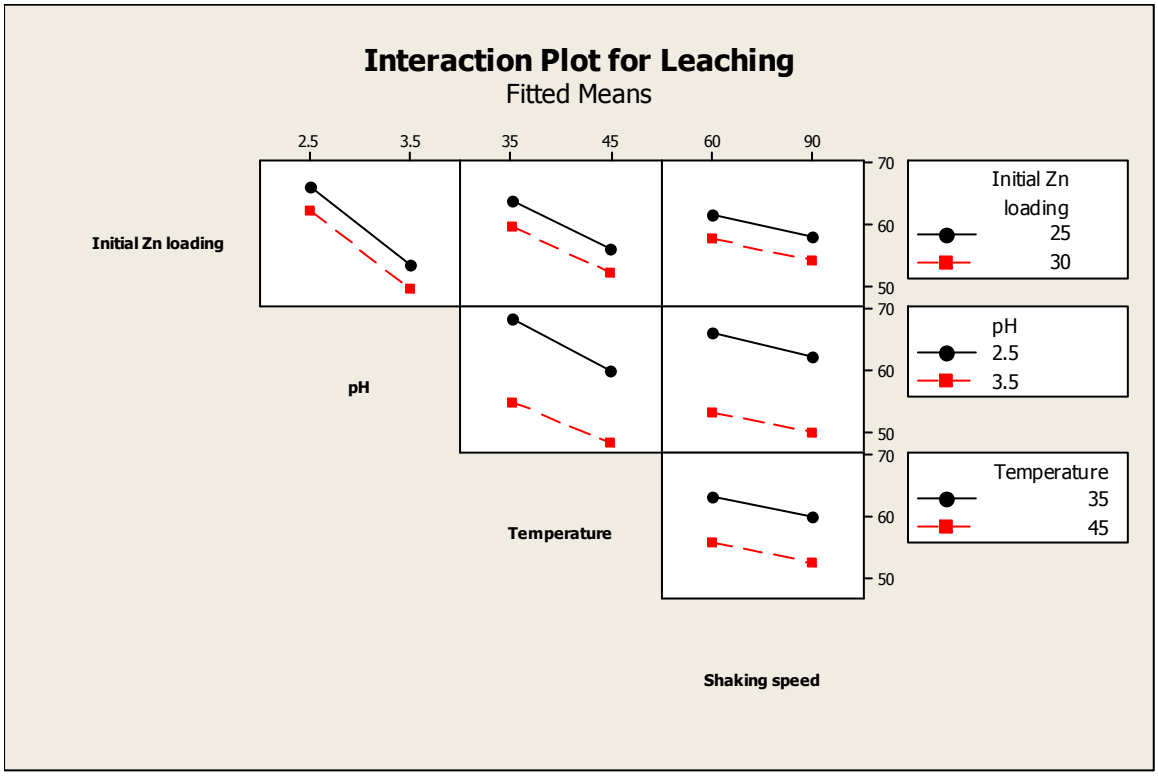

Figure 3.7 Interaction plot generated by MINITAB 15.

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Chapter 4

DISSOLUTION KINETICS OF NICKEL LATERITE ORE USING DIFFERENT SECONDARY METABOLIC ACIDS

4.1. INTRODUCTION

The characterization of secondary metabolite excreted by heterotrophs and proposition of efficient kinetic mechanism for laterite ore and secondary metabolite interaction adapting the shrinking core model; are the excerpts of chapter four. Remaining focused to the proposed objectivity; the present chapter presents a vivid documentation on resources of the strategic metal nickel; especially lean grade ores and overburdens, commercialization of nickel extraction by process routes apart from biological routes, heterotrophic leaching of nickel and its process design challenges with a mention of two-tank leaching, the theoretical postulation of shrinking-core model and its adaptation here to propose the dissolution kinetics of laterite ore in secondary metabolic acids, design and execution of model driven experiments to establish the kinetics and generation of design parameters extremely useful for modeling, simulation and control of this microbial process based on first principles.

Nickel is a strategic metal of vital importance in many modern industrial and metallurgical applications. Nickel is silvery-white, hard, malleable, and ductile metal. It is a fairly good conductor of heat and electricity. In its familiar compounds nickel is bivalent,

although it assumes other valences. It also forms a number of complex compounds. Most nickel compounds are blue or green. Nickel dissolves slowly in dilute acids but, like iron becomes passive when treated with nitric acid. Finely divided nickel adsorbs hydrogen. Nickel is the earth's 22nd most abundant element and the increasing world demand for nickel is reflected by its recent price increase.

Nickel is a very reactive element like other elements, but is slow to react in air at normal temperatures and pressures due to the formation of a protective oxide on its surface. Due to its permanence in air and its slow rate of oxidation, it is used in coins, for plating metals such as iron and brass, for chemical apparatus, and in certain alloys such as German silver. Nickel is chiefly valuable for the alloys it forms, especially many superalloys, and particularly stainless steel. Nickel alloys are characterized by strength, ductility, and resistance to corrosion and heat. About 65 % of the nickel consumed in the western world is used to make stainless steel, whose composition can vary but is typically iron with around 18% chromium and 8% nickel. 12 % of all the nickel consumed is for making super alloys. The remaining 23% of consumption is divided between alloy steels, rechargeable batteries, catalysts and other chemicals, coinage, foundry products, and plating. Nickel can be drawn into wire. It resists corrosion even at high temperatures and for this reason it is used in gas turbines and rocket engines. Monel is an alloy of nickel and copper (e.g. 70% nickel, 30% copper with traces of iron, manganese and silicon), which is not only hard but can resist corrosion by sea water, so that it is ideal for propeller shaft in boats and desalination plants. Nickel is also a naturally magnetostrictive material, meaning that in the presence of a magnetic field, the material undergoes a small change in length (AML-UCLA). In the case of nickel, this change in length is negative (contraction of the material), which is known as negative magnetostriction and is on the order of 50 ppm.

Most nickel on Earth is inaccessible because it is locked away in the planet's iron-nickel molten core, which is 10 % nickel. The total amount of nickel dissolved in the sea has been calculated to be around 8 billion tonnes. The nickel content in soil can be as low as 0.2 ppm or as high as 450 ppm in some clay and loamy soils. The average is around 20 ppm. Nickel occurs in some beans where it is an essential component of some enzymes. Another relatively rich source of nickel is tea which has 7.6 mg/kg of dried leaves.

The country's requirement of nickel is met fully through import of metal. Currently, India is importing annually about 4,000 tonnes of nickel and its associated products equivalent to about 10 crores of rupees. In terms of supply, the Sudbury region of Ontario, Canada, produces about 30 percent of the world's supply of nickel. Russia has about 40 percent of the world's known resources at the massive Norilsk deposit in Siberia. Russia mines this primarily for its own domestic supply and for export of palladium. Other major deposits of nickel are found in New Caledonia, Australia, Cuba, and Indonesia. Only known deposit in India is in Sukinda Valley, Orissa. To extract nickel from the chromite overburden generated during mining of chromite ore in Sukinda Valley, a process route was developed jointly by the Department of Mines and the Council for Scientific and Industrial Research (CSIR). To verify the critical parameters of the process, a 10 tonnes per day ore throughput pilot plant was set up at Institute of Minerals and Materials Technology (IMMT), Bhubaneswar at a cost of Rs10.5 crore jointly by HZL and CSIR. The expenditure was shared equally. The technical feasibility of extracting nickel from COB has been established. Nowhere in the world has nickel with such a low concentration (0.6%) been extracted from similar oxidic ores. HZL has transferred its first right to the use of technology to National Aluminium Company Limited. The actual expenditure incurred by HZL will be reimbursed by NALCO. NALCO will proceed further to establish the techno-economic viability of the process.

The underground mining technology to recover deposits below 65m in depth in chromites mines in the Sukinda Valley in Orissa is the major reserve of India's lateritic deposit is in, estimated to be 65 million tons with 0.15-1.2% nickel content (Sukla, et *al.,* 1987, Kanungo, et *al.,* 1988). The only significant deposit of lateritic nickel ore in India, which is in the ultra basic belt of Sukinda, is yet to be commercially exploited.

Nickel ores are of two types: sulphide and oxide or lateritic (saprolite, non-tronite, and limonite). Nickel is commonly found in iron meteorites as the alloys kamacite and taenite. Nickel deposits comprise two main sub-types (Valix et *al.,* 2001):

a) Primary sulphide deposits associated with mafic and ultramafic rocks

b) Near-surface laterite deposits formed over olivine-rich host rocks following intense weathering.

The sulphide ores have been the major source of nickel to date, however the lateritic ores have been estimated to constitute about 73% (Nickel Market Overview-The supply Response, prepared for INSG Meeting, October 2006, Prepared by Vanessa Davidson, CRU Special Steels & Alloys Team) of the known nickel reserves of the world. Laterites are important as hosts to economic ore deposits, as the chemical interactions which together make up the lateritisation process can in certain cases be very efficient in concentrating some elements. Several wastes are created when minerals are mined from the earth. The first is overburden, which is soil and removed in order to access an ore or mineral body. The huge amount of overburden (nearly 8 to 10 times of the ore) which is generated during chromite mining and dumped nearby, has found a very little use so far. With the continuous depletion of high grade nickel sulphide ores, there is the need to win metals from the abundant low grade nickel laterite ores and overburdens as well.

4.1.1. Microbial Leaching of Nickel Laterites

Bioleaching involves the utilization of microorganisms and their metabolic products to dissolve nickel from low grade nickel laterite ores. Both autotrophic (Torma and Bosecker, 1982) and heterotrophic (Kiel and Schwartz, 1980; Sukla and Panchanadikar, 1983; Munier-Lamy and Berthelein, 1987) microorganisms possess the potential of nickel removal from their ores.

For sulfidic ore leaching, *Thiobacillus ferrooxidans* and *Thiobacillus thioxidans* were widely used organisms. Bioleaching mechanisms of sulphidic minerals using heterotrophs have received less attention from microbiologists (Kiel and Schwatz, 1980). Among the heterotrophic bacteria, members of the genus *Pseudomonas* have been found to be effective in the leaching of non sulphidic minerals (Karavaiko et *al.,* 1998). Fungi of the genera *Penicillium* and *Aspergillus* have also been used in mineral leaching (Ehrlich and Rossi, 1990).

Non-sulphidic ores do not contain any energy source for the microorganism to utilize. Such ores can be leached out by heterotrophic bacteria and fungi which require a carbon source for their energy supply and growth. Fungi are expected to be efficient bioleachers due to their

acid secreting potentials. The organism utilizes the carbon source and excretes organic acids and compounds with at least two hydrophilic reactive groups (e.g. phenol derivatives) into the culture medium as metabolic products. The secondary metabolites that are produced by the heterotrophic organisms from the organic carbon they consume for energy production, interacts with the mineral surfaces. In addition to forming several organic acids such as acetic acid, citric, oxalic and α-ketoglutaric acid (Agatzini and Tzeferis, 1997; Castro, 2000; Natarajan and Deo, 2001), they also produce exopolysaccharides (Malinovskaya et *al.,* 1990; Welch and Vanderivere, 1995; Welch et *al.,* 1999), amino acids and proteins that can solubilize the metals via a variety of mechanisms (Henderson and Duff, 1963; Avakyan and Robotnova, 1971; Valix et *al.,* 2001b). Organic acids however disseminate a central role in the overall process supplying both protons and metal complexing organic acid anions (Gadd, 1999).

In this context, in 2001, Ehrlich proposed a two-reactor leaching process for heterotrophic leaching in which the first reactor would be a generator, where the desired microbes would produce the acids in pure culture under optimal growth conditions. The spent culture solution from this reactor would be bleed into a second reactor, where the maintenance of sterile conditions is not required, containing the ore to be leached. He also suggested that the growth of microbes due to contamination on the ore, which might destroy the acids, could be controlled by ensuring a very low level of residual nitrogen source in the spent culture medium as nitrogen source being essential for growth, and by temperature manipulation. In the second reactor the leaching process becomes a hydrometallurgical process rather a bio-hydrometallurgical one. Here the dissolution kinetics plays an important role in optimizing the process and making it more economical.

The present study was concentrated on the second reactor of two stage process as optimal growth conditions for different heterotrophic microorganism has been already discussed in chapter 2. As the organic acids produced by microorganism were playing the vital role in the heterotrophic leaching of nickel, current studies have been undertaken to study the leaching behavior of organic acids on nickel laterite ore. An FTIR analysis has been done for the secondary metabolite solution of heterotrophic bacteria *Pseudomonas putida* to confirm the presence of phenol and its derivatives. A hypothesis that the presence of phenol and its derivatives might have some effect on the leaching process was tested. The dissolution

mechanism and kinetics of laterite ore leaching with the possible organic acids present in the secondary metabolite; concluded on the basis of FTIR analysis and available literature support has been a major aim of this study. An attempt has been made in laboratory scale reactor to test all the possible mechanisms of the dissolution kinetics and proposition of a suitable one for the present case.

4.2. REVIEW OF LITERATURE

Some of the pioneer research works regarding nickel extraction through bioleaching are compiled as follows: Roorda, 1984, reported the recovery of nickel and cobalt from limonite. Nickeliferrous limonite constitutes by far the largest known terrestrial reserves of nickel and cobalt. They are also major future sources of chromium and iron. He attempted to render some guidelines for the future studies. Sukla and Das, 1986, reported the leaching behavior of Sukinda lateritic nickel ore with sulphuric acid. Mineralogically, the Sukinda ore contains goethite, quartz, chromite and manganese in oxide form in the iron matrix and not as a separate mineral. They have also reported that at about 360^{o}C goethite decomposes to hematite. McKenzie et *al.* (1987) studied on the solubilization of nickel, cobalt and iron from laterites by means of organic chelating acids at a low pH. Sukla et *al.* 1993 reported that 90% of the nickel and 34% of the cobalt present were extracted from the reaction of laterite and organic acid such as oxalic acid and citric acid and maleic acids generated by microbial metabolism. The percentage of dextrose in the growth medium was varied from 2% to 10% in order to increase the nickel extraction. Sukla et. *al.* 1995 reported on increased ability of *Aspergillus niger,* a fungal strain in nickel leaching. They achieved 95% nickel leaching with ultrasound pre-treated strains of *Aspergillus niger* in 14 days as compared to 92% nickel leaching after 20 days with untreated *Aspergillus niger*.

There are certain mechanisms proposed by different researchers; such as reduction, complexolysis, chelation, have been proved to be instrumental in metal solubilization. Minerals such as limonite, goethite, or hematite can be solubilized by certain microorganisms through reduction (Ehrlich, 1986; Ferris et *al.,* 1989). Microbial oxidation of organic compounds may produce non-complexing or weak complexing acids (carbonic, nitric, sulphuric, formic, acetic, butyric, lactic, succinic, gluconic acid etc.). Complexolysis is a process that utilizes microbially

formed complexing agents that mobilize metal constituents (Fe, Al, Cu, Zn, Ni, Mn, Ca, Mg, etc.) (Beveridge, 1989). Fermentation and degradation of organic macromolecules by microbes results in the production and excretion of organic ligands (Berthelin, 1983; Gadd, 1999; Gottschalk, 1986; Welch and Ullman, 1999). These ligands can increase the rates of mineral dissolution by chelation (Amrhein and Surez, 1988; Wieland et *al.,* 1988).

Microbial extra cellular polysaccharides are also produced by microbes, that can enhance mineral dissolution by complexing with ions in solution, or they can inhibit dissolution by irreversible binding to reactive sites on the mineral surface (Welch and Ullman, 1999; Welch and Vandevivere, 1995). It can be concluded that, microorganisms are able to mobilize metals by (i) formation of organic acid, (ii) oxidation-reduction reactions, (iii) extraction by complexity agents, (iv) chelate formation.

Mineral leaching with heterotrophs poses some process design challenges, which autotrophs do not. Two-stage process is becoming a convenient process for handling heterotrophic leaching. Groudev (1987), developed a process for biological removal of iron from quartz sands, kaolins and clays in which these industrial minerals were leached at 90°C with lixiviant produced as a result of the cultivation of acid-producing heterotrophic microorganisms, mainly strains of *Aspergillus niger*, at 30°C in a nutrient medium containing molasses as a source of carbon and energy. This was almost the first reported two-stage leaching process using heterotrophs.

Cameselle et al (2003) have compared the in-situ and two-stage bioleaching process using *Aspergillus niger* and showed that two-stage approach is better than in-situ approach. They also discussed some of the draw backs of in-situ leaching. They are:

- Filamentous fungi as *A. niger* may be affected by low concentrations of heavy metals, inhibiting growth and metabolic activity, mainly due to four factors: blocking of enzymes, displacement of essential metals, induction of conformational changes in polymers and influence on membrane integrity and transport processes (Gadd, 1990). These factors can reduce largely the extent of metal removal.

- The metal dissolution rate is lower than in the two-stage technique, and it cannot be improved by increasing temperature (or by altering other variables) because of the damage that can be caused to the microorganism.
- The mineral is adsorbed in biomass, thus, making it difficult to recover.
- The mineral treated should be sterilized to prevent the proliferation of undesirable microorganisms.

Leaching kinetics plays an important role in the extraction of metals and their compounds in an economical way. Many researchers have studied the dissolution kinetics in hydrometallurgy (Abdel-Aal, 2000; Antonijevi et *al.,* 1997; Ekinci et *al.,* 1998; Liang et *al.,* 2005; Raschman and Fedorockova, 2004). Inorganic acids have been commonly used as leach reagent in these studies. In addition to these, organic acids are in use for that purpose in the recent years (Bakan et *al.,* 2006; Bayrak et *al.,* 2006; Demir et *al.,* 2006; Fred and Fogler, 1998). Ingec et al. (2004) have conducted the dissolution kinetics study on smithsonite ore (ore of zinc) with an organic leach reagent in the presence of ultrasound energy and it had a positive effect on dissolution rate. In case of nickel most of the dissolution studies have been done on leaching of spent catalyst (Mulak et *al.,* 2005; Mishra et *al.,* 2008). Mishra et al. (2008) has studied the dissolution kinetics of spent catalyst using acidophilic bacteria. Scheckel et al. (2001) studied the dissolution kinetics of nickel surface precipitates on clay mineral and oxide surfaces using an array of dissolution agents like EDTA, oxalate, acetyl acetone and HNO_3.

Alkan et al. (2004) attempted the study of dissolution kinetics of colemanite ore in oxalic acid solution. The dissolution kinetics of nickel laterite ore has been studied by Landers et al. (2009). In their study they used sulfuric acid as leaching agent. It was reported by them that there was a 9–34-fold increase in the dissolution rate constant (k) for samples heated at 340–400 °C due to both the increased surface area (1.5–2.6 fold) and higher density of structural defects (5–10 fold) in the variously dehydroxylated products. Although Tang et al. (2006) have studied the nature of nickel dissolution from limonite and notronite ore using secondary metabolic acids of heterotrophic microorganisms, nobody has attempted to study the effect of other metabolites (like phenol and phenyl derivatives) that are present in secondary metabolites on the leaching process of nickel.

On the basis of the aforesaid background, certain objectivities were chosen for the present work, which are as follows,

An FTIR analysis has been conducted to ascertain an idea about different functional moieties present in the secondary metabolite solutions of *P.putida*. After confirming the presence of phenol and its derivatives, an attempt has been made to evaluate their effect on nickel leaching process.

Further, the dissolution kinetics has been studied on nickel laterite ores using three different organic acids, which are found in secondary metabolites of heterotrophs. As no literature supports; till date were found regarding this, the objective was to propose a suitable mechanism for the dissolution of lateritic ore particles when leached with secondary metabolic acids.

4.3. REACTION MECHANISM AND DISSOLUTION KINETICS

The dissolution of lateritic ore particles with metabolic acids like citric acid, oxalic acid, acetic acid falls under the category of heterogeneous reaction, i.e., the reaction handling more than one phase (solid-liquid here). Since more than one phase is present, the movement of material from phase to phase is to be considered in the rate equation. Thus the rate expression, in general, will incorporate mass transfer terms in addition to the usual chemical kinetics term. These mass transfer terms are different in type and numbers in the different kinds of heterogeneous systems; hence, no single rate expression has a broad generality and applicability. The rate expression is also unique depending on the contacting pattern of the phases.

In the present study the liquid phase (Citric acid, Oxalic acid) contacts with solid ore and under the experimental condition maintained the products are the fluid products. The solid particle undergoing reaction remain unchanged in size during the course of reaction because the host particle bearing the reactant nickel is predominantly hydrated iron oxide or goethite (FeOOH) that remains unaffected by the organic acid concerned. The goethite, when roasted before leaching, is converted to hematite. The hydrated iron oxide, hematite, remain inert while exposed to organic acids/secondary metabolites and may be considered as non flaking ash here. With time, the reactive core of nickel oxide diminishes leaving behind the inert solid or ash.

Thus at any point of time there exists an unreacted core of reactant nickel oxide, which shrinks in size during reaction as shown in fig. 4.1. The following are the steps adapted here from the *shrinking core* model, originally proposed by Yagi and Kunii (1955, 1961) to simulate the reaction between the ore particle and the acids.

Step1. Diffusion of the liquid reactant through the film surrounding the particle to the surface of the solid.

Step2. Penetration and diffusion of the liquid reactant through the blanket of ash to the surface of the unreacted solid reactant core.

Step 3. Reaction of the liquid reactant at the surface of the solid reactant.

In developing a conversion equation for the ore particle, considered spherical here; either of the steps 1, 2 & 3 may be the rate controlling steps. It is useful to express the reaction rate in terms of fraction reacted (i.e. particle conversion) (Chae, 1979; Burghardt, 2001; Liddell, 2005). Much attention had been given on application of shrinking core model regarding the ore leaching with organic and inorganic acids for the last few years. It is important to notice that the models involves the pseudo-steady state (PPS) approximation (Liddel, 2005), hence, the determination of the reactant concentration becomes a possibility across the porous ash layer.

For a spherical particle (fig. 4.2) involving the quasi-steady state diffusion of the reactant through the ash layer of the particle, followed by the chemical reaction at the surface of the unreacted core, the shrinkage process, the fraction reacted (α) and the reaction time (t) are related for different control regimes.

If the process is controlled by the reactant diffusion through the fluid surrounding the particle, the derived integral rate expression, eq. (4.1) becomes the governing equation.

$$\frac{t_f}{\tau} = 1 - \left(\frac{r_c}{R}\right)^3 = \alpha \qquad (4.1)$$

Where, r_c is the unreacted core radius at given time, and R is the initial particle radius. τ is the time required for the particle to react completely, i. e., $r_c = 0$.

$$\tau = \left(\frac{\rho_p R}{3bk_l C_A} \right) \tag{4.2}$$

Where, b is the stoichiometric coefficient of solid particle, ρ_p is the molar density of the reactant nickel oxide in solid B, k_l is the mass transfer coefficient for the liquid film, and C_A bulk concentration of the liquid (acid here).

If the reaction is controlled by ash layer diffusion, the derived integral rate expression eq. (4.3) becomes the governing equation

$$\frac{t_a}{\tau_a} = 1 - 3\left(\frac{r_c}{R} \right)^2 + 2\left(\frac{r_c}{R} \right)^3 = 1 - 3(1-\alpha)^{\frac{2}{3}} + 2(1-\alpha) \tag{4.3}$$

τ_a is the time required for the particle to react completely, i. e., $r_c = 0$.

$$\tau = \left(\frac{\rho_p R^2}{6bD_e C_A} \right) \tag{4.4}$$

Where, D_e is the effective diffusion coefficient through the ash layer.

When the process is controlled by the chemical reaction at the surface of the ore, the derived integral rate expression, eq. (4.5) governs

$$\frac{t_{ch}}{\tau_{ch}} = 1 - \left(\frac{r_c}{R} \right) = 1 - (1-\alpha)^{\frac{1}{3}} \tag{4.5}$$

τ_{ch} is the time required for the particle to react completely , i. e., $r_c = 0$.

$$\tau_{ch} = \frac{\rho_p R}{b k_s C_A} \tag{4.6}$$

Where, k_s is the rate constant for the surface reaction. The conversion of nickel oxide with respect to time along with physical properties of solid, stoichiometric coefficient of solid particle in the concerned reaction, and the molar density of the liquid enable us to determine, the valuable parameter D_e/effective diffusion coefficient for shapes other than spherical. There are different ways to find out the mechanism that controls (eq. 4.1, 4.3 & 4.5) the shrinkage process. Conversion- time equations similar to those developed above can be obtained for various shaped particles, and Table 4.1 summarizes these equations.

Experiments using different particles radius (R_1, R_2) are carried out, for different times (t_1, t_2) necessary to attain the same particle conversion. The following design equations hold:

In the case of the reactant diffusion through the fluid surrounding the particle controls,

$$\frac{t_1}{t_2} = \left(\frac{R_1}{R_2} \right)^{1.5-2.0} \tag{4.7}$$

If the process is controlled by ash layer diffusion:

$$\frac{t_1}{t_2} = \left(\frac{R_1}{R_2} \right)^{2.0} \tag{4.8}$$

If the process is chemical reaction controlled:

$$\frac{t_1}{t_2} = \left(\frac{R_1}{R_2} \right) \tag{4.9}$$

In hydrometallurgy the shrinking core models are generally applied to describe the shrinkage of ore particles during mineral leaching reactions, which are a central unit operation in the hydrometallurgical ores treatment. The model is applied to description of the leaching process both for column or heap leaching (Lizama, 2004, Chae, 1979) and for stirred-tank

reactors, including continuous systems (Crundwell, 1995). Literature supports are also available on the use of the shrinking core model for modeling of bacterial leaching processes of nickel / zinc sulphide ores (Brochot, 2004; Conner, 2005, Leachy, 2005). In view of this, the present work is focused on proposition of heterogeneous reaction mechanism between nickel laterite ore and organic acids present in secondary metabolite of heterotrophs.

4.4. MATERIALS AND METHODS

4.4.1. FTIR Analysis of Secondary Metabolite Solution

The secondary metabolite solutions of *Pseudomonas putida* (NCIM-2650) was collected after an incubation period of 2 days. The micro organism was obtained from NCIM, Pune. The broth culture of *P.putida* was collected by vacuum filtration as it is difficult to separate the bacteria through normal filtration and centrifugation methods. The strains were inoculated in Bromfield media which contains higher levels of sucrose. In the absence of sufficient amount of nitrogen source; this excess amount of sucrose will be used in production of metabolic acids rather than producing the biomass. The composition of Bromfield media used was as follows:

$(NH_4)_2SO_4$	0.25g/L
KH_2PO_4	0.25 g/L
$MgSO_4$	0.7 g/L
Sucrose	20 g/L
Yeast extract	1.0 g/L
PH	6.5

The FTIR analysis was done with the collected secondary metabolite solution.

4.4.2. Ore Material Source

The nickel ore used for the study was overburden of chromite mine and was obtained from the major deposits of Sukinda mines, Orissa, India. The sample was collected from the site of Orissa Mines Corporation.

4.4.3. Chemical Analysis of Ore

The acidified raw ore overburden was subjected to chemical analysis to determine the percentage of nickel. One gram of raw ore was taken and was added to 50ml of concentrated, hydrochloric acid (HCL) in beakers and then the mixture was heated until the residue turned white. The mixture was cooled and filtered and kept in a clean volumetric flask, which was rinsed prior with distilled water for several times. Then the volume was made up to 250ml by adding distilled water. Then the solution was filtered and the solution was diluted 10times, 100times and 1000times for analysis of nickel metal by Atomic Absorption Spectrophotometer (AAS). An XRF analysis has been done to find all the metal constituents present in the laterite ore.

4.4.4. Mineralogical Analysis of Ore

Mineralogical analysis of the nickel laterite samples was done by using high-resolution synchrotron based X-ray diffractometer (XRD).

4.4.5. Effect of Phenol on Leaching

Leaching tests were conducted in 250ml conical flasks using analytical reagent grade citric, oxalic and acetic acid. Three mixed acid solutions of overall concentrations of 1.5, 2.1, 3 M were prepared by taking equimolar amounts of citric, oxalic and acetic acid. To enquire the effect of phenol on leaching process three phenol concentrations of 10%, 20% and 30% were added for each concentration level of mixed acid solution. The pulp density was maintained at 10 gm/L in the reaction flasks. These flasks were incubated in a shaker incubator at 60 rpm and 45^0C. The reason for maintaining the temperature at high levels is that the melting point of pure phenol is about 38^0C. The incubation period was 10 days. After incubation each sample was centrifuged at 4000 rpm for 5 min to remove the ore.

The overall distribution of samples is as follows:

	10%Phenol (1)	20%Phenol (2)	30%Phenol (3)
1.5 M Mixed acid (A)	A1	A2	A3
2.1 M Mixed acid (B)	B1	B2	B3
3.0 M Mixed acid (C)	C1	C2	C3

After the incubation all the leached solutions were analysed for the estimation of nickel using Atomic Absorption Spectroscopy (AAS).

4.4.6. Study of Dissolution Kinetics

For the study of dissolution kinetics three experiments have been conducted in an indigenously designed laboratory scale batch rector (fig. 4.3) process with three different acids. The batch reactor is of 6 litter capacity and a pulp density of 10 gm/L was maintained in the reactor. 1 M acid concentration was used for the leaching experiments and the experiments were done with three individual acids namely oxalic acid, citric acid and acetic acid. The temperature of the reactor was maintained at 32^0C. 5ml sample was collected from the reactor after every 8hrs and was centrifuged at 4000 rpm for 5min to remove the ore particles. The supernatant leached solution was taken for AAS analysis to determine the nickel concentration. The process flow diagram (PFD) is presented in fig. 4.4.

4.5. RESULTS AND DISCUSSION

4.5.1. FTIR analysis of Secondary Metabolite Solution

Fig. 4.5 shows the FTIR analysis of secondary metabolite solution collected from *Pseudomonas putida*. It clearly indicates the presence of carboxylic acid groups (peaks in the ranges of 1760-1670 cm^{-1} (C=O stretch) and 1395-1440 cm^{-1} (C-O-H bend)). The weak peaks in the range of 2000-1600 cm^{-1} and 3600-3200 cm^{-1} are indicative of the presence of phenols. With the help of the FTIR analysis and the literature support, the presence of oxalic acid, citric acid, acetic acid and phenol were concluded in the secondary metabolite solution of *Pseudomonas putida* and the effect of different acids and phenol on the conversion of nickel was studied.

4.5.2. Mineralogical Analysis of Ore

The XRD study revealed the presence of goethite, hematite, nickel ferrite, garnierite and quartz in the ore body. The mineralogical studies indicated that there is no separate nickel bearing mineral phase in the lateritic nickel ore. Goethite or hydrated iron oxide is the main iron bearing phase or host which contains most of the nickel oxide in the raw lateritic ore.

4.5.3. Chemical Analysis of Ore

The chemical analysis of raw lateritic ore has revealed the presence of 0.97 % of Ni along with some other different metals as confirmed by the XRF study of nickel ore (Table 4.2).

4.5.4. Effect of Phenol on Leaching

The percentage leaching of nickel by the mixed acid of different molarities (1.5, 2.1 and 3.0 M) in the presence of three different concentrations of phenol (10%, 20%, 30%) are shown in the Fig. 4.6. It is evident that as the phenol concentration got increased the percentage leaching of nickel decreased. This may be attributed to the esterification process that takes place between the carboxylic acids and alcohols which increases the pH of the solution, hence, resulting in decreased leaching efficiency of the mixed acids.

4.5.5. Effect of Acids on Leaching

Fig. 4.7 shows the percentage leaching of nickel for different acids at a time interval of 40hrs. From the figure it is being reflected that the oxalic is the most efficient leaching agent compared to other two acids.

4.5.6. Dissolution Kinetics of Nickel Oxide Present in Laterite

The reaction of laterite ore can proceed via the shrinkage of the inner reactive core of the particle with time. Leaching kinetics are controlled either by the diffusional mass transfer of the reactant through a liquid boundary layer or ash layer or chemical reaction at the ore surface. The reaction between nickel oxide, captive in goethite core and organic acids were analyzed by using heterogeneous reaction mechanism. The fit of the experimental data into the integral rate

equations (Table 4.1) was tested by using linear regression and the regression coefficients obtained for the three integral rate expressions for three different acids were calculated. The calculated regression coefficients controlled by different regimes are being presented in Table 4.3. The highest regression coefficients or the best fits for all the experimental results concerning ore leaching using different acids were obtained for rate expressions controlled by diffusion through ash layer. Fig. 4.8 shows the goodness of fit of the experimental data using integral rate expression (eq. 4.3), which is governed by ash layer diffusion. From the slopes of those best fit curves of our experimental results to the integral rate expression pertaining to ash layer diffusion control, the apparent rate constant of the reactions between ore particles and organic acids were found. The effective diffusion coefficients for the diffusion of liquid reactants through the ash layer for spherical ore particle were calculated using the slope of the best fit curves of fig. 4.8 the Sauter Mean Diameter (SMD) of the ore particles, the molar density of nickel oxide, and the stoichiometric coefficient of reactant nickel oxide in the concerned reaction. The particle diameter (SMD) is calculated by using the sieve analysis results of the ore particles. The calculated effective diffusion coefficients are as follows:

Citric Acid $D_e = 1.98567 \times 10^{-9}$ cm^2/s

Oxalic Acid $D_e = 2.5907 \times 10^{-8}$ cm^2/s

Acetic Acid $D_e = 1.91904 \times 10^{-10}$ cm^2/s

On the basis of those D_e values obtained, the oxalic acid evolved out to be the prime mover in heterotrophic leaching of nickel laterite ore.

4.6. CONCLUSION

The mineralogical studies indicated that there was no separate nickel bearing mineral phase in the lateritic nickel ore. Goethite is the main iron bearing phase or host which contains most of the nickel in the raw lateritic ore. This study also revealed the presence of hematite, nickel ferrite, garnierite and quartz in laterite ore. With the help of the FTIR analysis and the literature support, the presence of oxalic acid, citric acid, acetic acid and phenol were confirmed in the secondary metabolite solution of *Pseudomonas putida* and the effect of different acids and phenol on the conversion of nickel was studied. Presence of phenol in the reaction media had a

negative effect in leaching process and it could be attributed to the esterification of the organic acids. The dissolution of lateritic ore particles with metabolic acids like citric acid, oxalic acid, acetic acid falls under the category of heterogeneous reaction. The reaction between laterite ore and organic acids were ash layer diffusion controlled. The effective diffusion coefficients obtained could be immensely useful for developing first principle models for simulation, control and scale-up of such a microbial process.

Table 4.1 Conversion-time equations for various shapes of particles in shrinking core model

Shape of the particle	Film diffusion controls	Ash diffusion controls	Reaction controls
Flat plate $\alpha = 1 - \frac{1}{L}$ L=half thickness	$\frac{t}{\tau} = \alpha$ $\tau = \frac{\rho_p L}{b k_l C_A}$	$\frac{t}{\tau} = \alpha^2$ $\tau = \frac{\rho_p L^2}{2 b D_e C_A}$	$\frac{t}{\tau} = \alpha$ $\tau = \frac{\rho_p L}{b k^n C_A}$
Cylinder $\alpha = 1 - \left(\frac{r_c}{R}\right)^2$	$\frac{t}{\tau} = \alpha$ $\tau = \frac{\rho_p R}{2 b k_l C_A}$	$\frac{t}{\tau} = \alpha + (1-\alpha)\ln(1-\alpha)$ $\tau = \frac{\rho_p R^2}{4 b D_e C_A}$	$\frac{t}{\tau} = 1 - (1-\alpha)^{1/2}$ $\tau = \frac{\rho_p R}{b k^n C_A}$
Sphere $\alpha = 1 - \left(\frac{r_c}{R}\right)^3$	$\frac{t}{\tau} = \alpha$ $\tau = \frac{\rho_p R}{3 b k_l C_A}$	$\frac{t}{\tau} = 1 - 3(1-\alpha)^{2/3} + 2(1-\alpha)$ $\tau = \frac{\rho_p R^2}{6 b D_e C_A}$	$\frac{t}{\tau} = 1 - (1-\alpha)^{1/3}$ $\tau = \frac{\rho_p R}{b k^n C_A}$

Table 4.2 Analysis of Iron Ore (XRF).

Weight Percentage (%)					
Ni	**Co**	**Fe**	**Cr**	**Mn**	**Acid insoluble (AI)(gm)**
0.97	0.032	46.86	4.14	0.37	0.88

Table 4.3 Kinetic equations for different mechanisms and their regression coefficients for linearity.

Kinetic Equation	Regression Coefficient		
	Citric Acid	**Oxalic Acid**	**Acetic Acid**
Film diffusion controls (any shape) and reaction controls (flat plate) $\frac{t}{\tau} = \alpha$	0.8924	0.9049	0.8683
Ash layer diffusion controls (flat plate) $\frac{t}{\tau} = \alpha^2$	**0.971**	**0.945**	**0.959**
Ash layer diffusion controls (cylindrical) $\frac{t}{\tau} = \alpha + (1-\alpha)\ln(1-\alpha)$	**0.972**	**0.949**	**0.96**
Ash layer diffusion controls (spherical) $\frac{t}{\tau} = 1 - 3(1-\alpha)^{2/3} + 2(1-\alpha)$	**0.973**	**0.954**	**0.960**
Reaction controls (spherical) $\frac{t}{\tau} = 1 - (1-\alpha)^{1/3}$	0.8831	0.928	0.8997
Reaction controls (cylindrical) $\frac{t}{\tau} = 1 - (1-\alpha)^{1/2}$	0.8978	0.912	0.8697
First order reaction $\frac{t}{\tau} = -\ln(1-\alpha)$	0.9032	0.9187	0.871
Second order reaction $\frac{t}{\tau} = \alpha/(1-\alpha)$	0.9135	0.9312	0.8737

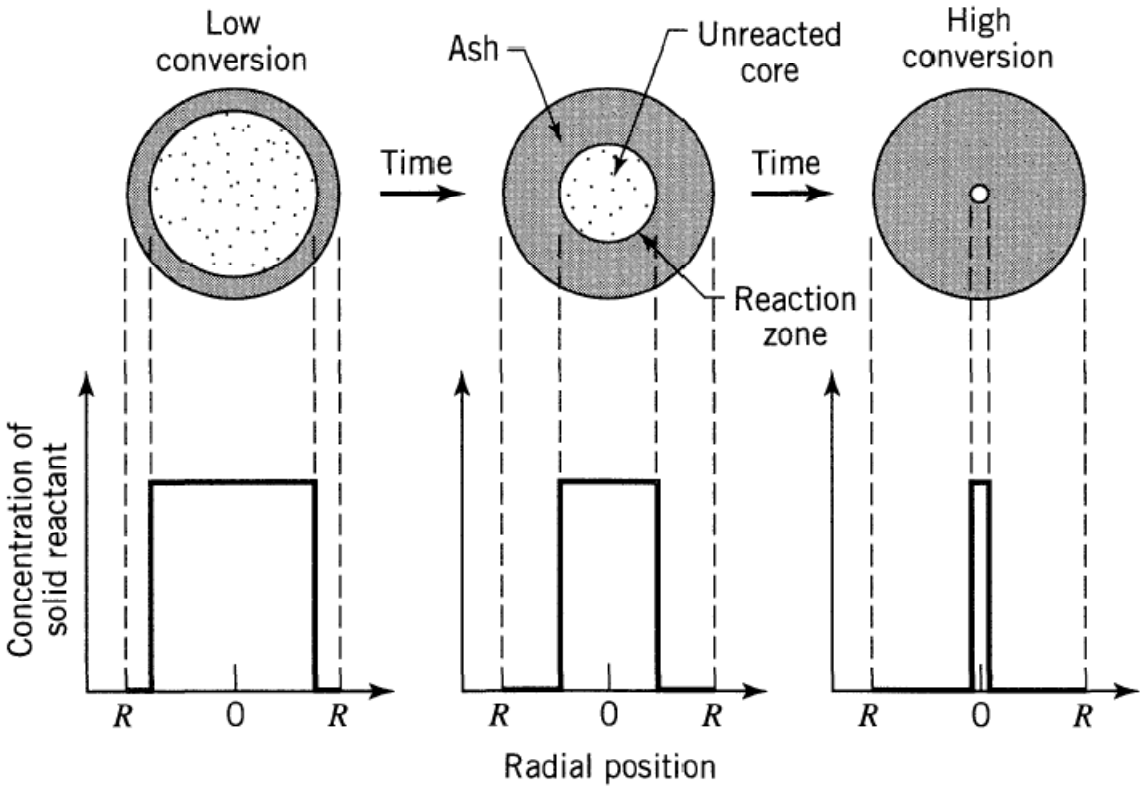

Figure 4.1 Shrinking of the un-reacted core during the reaction.

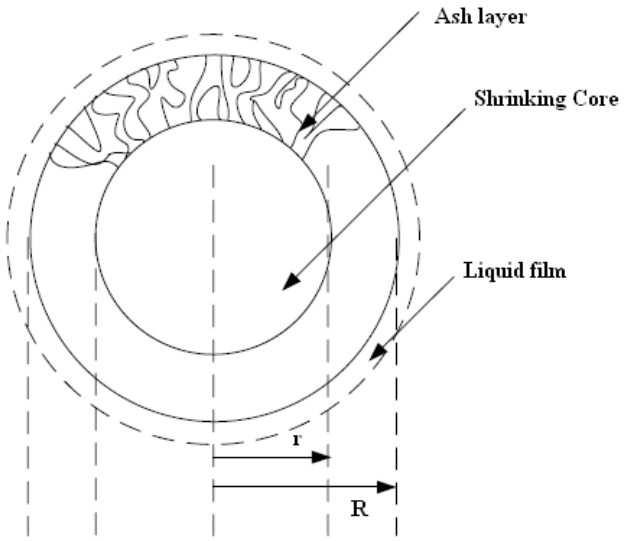

Figure 4.2 Shrinking core particle.

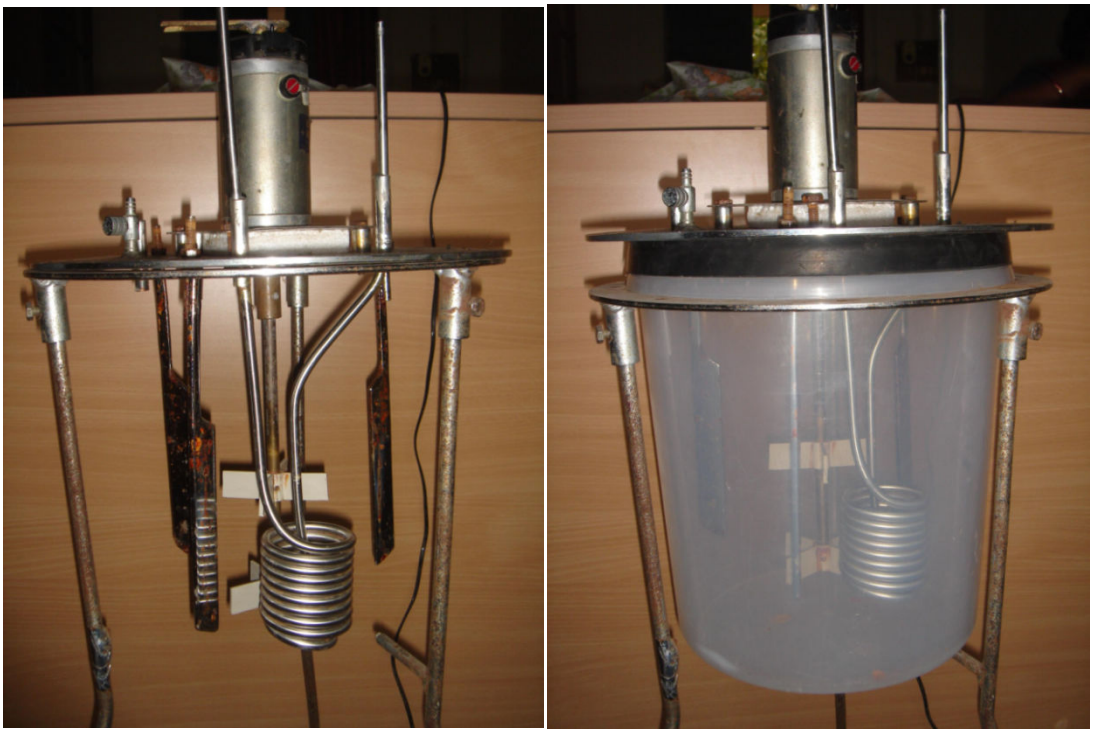

Figure 4.3 Laboratory scale bioreactor used for leaching experiments

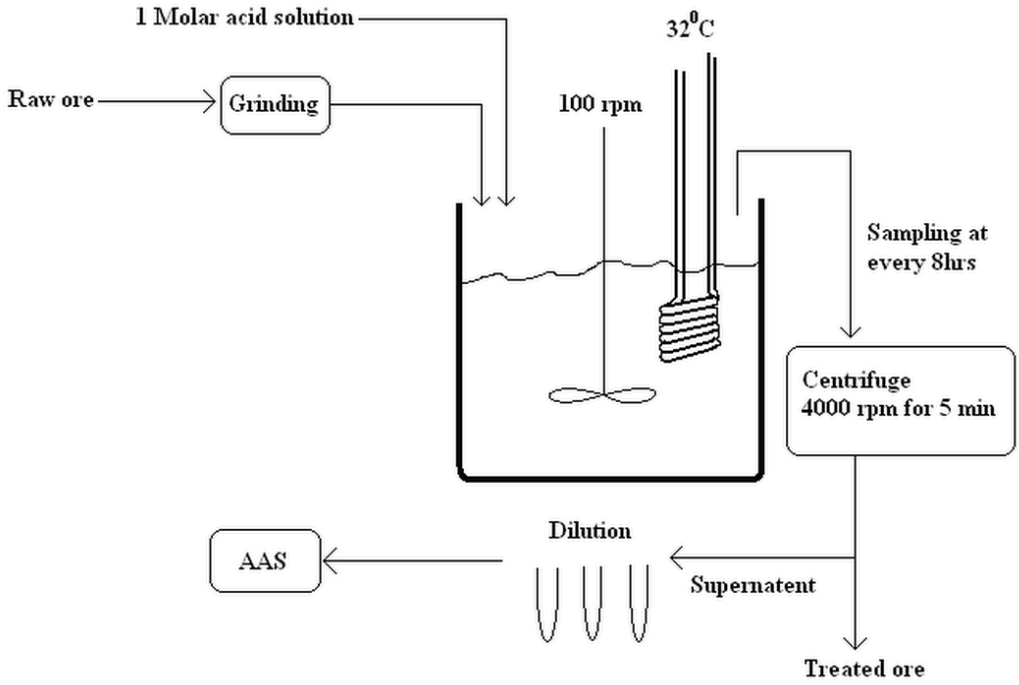

Figure 4.4 Process flow diagram for the study of dissolution kinetics of nickel oxide.

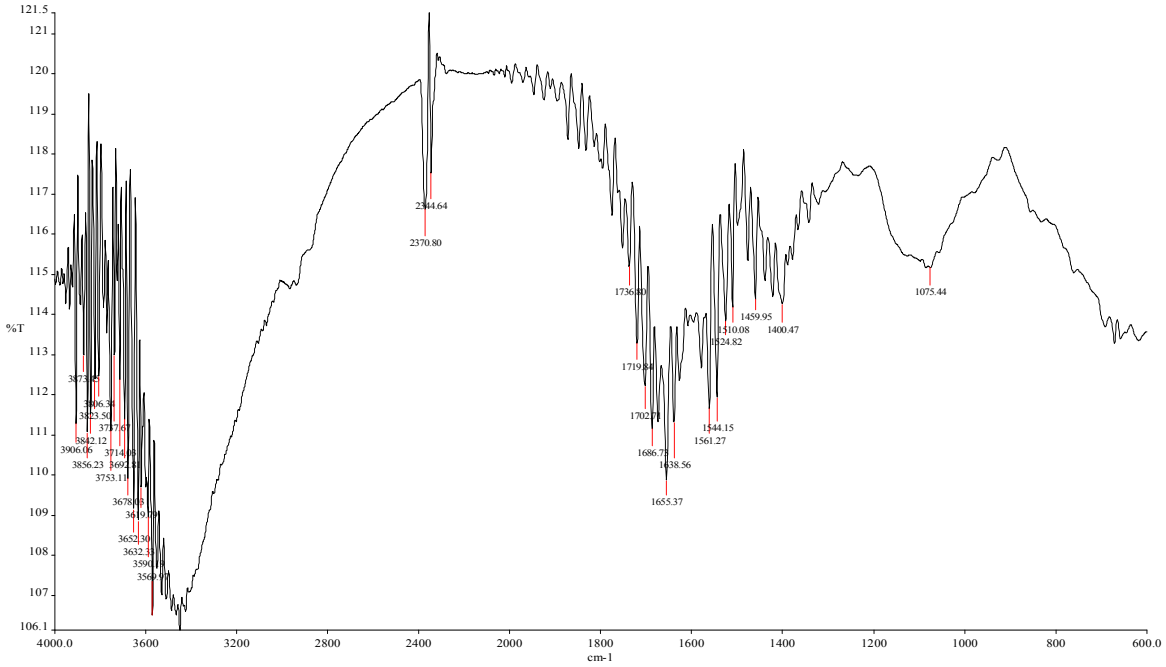

Figure 4.5 FTIR analysis of secondary metabolite solution of *Pseudomonas putida.*

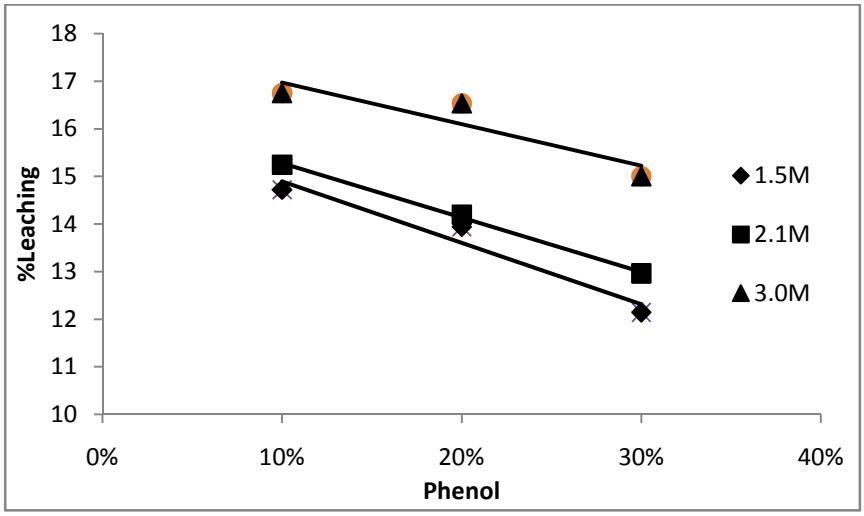

Figure 4.6 Effect of phenol on nickel leaching at different mixed acid concentrations

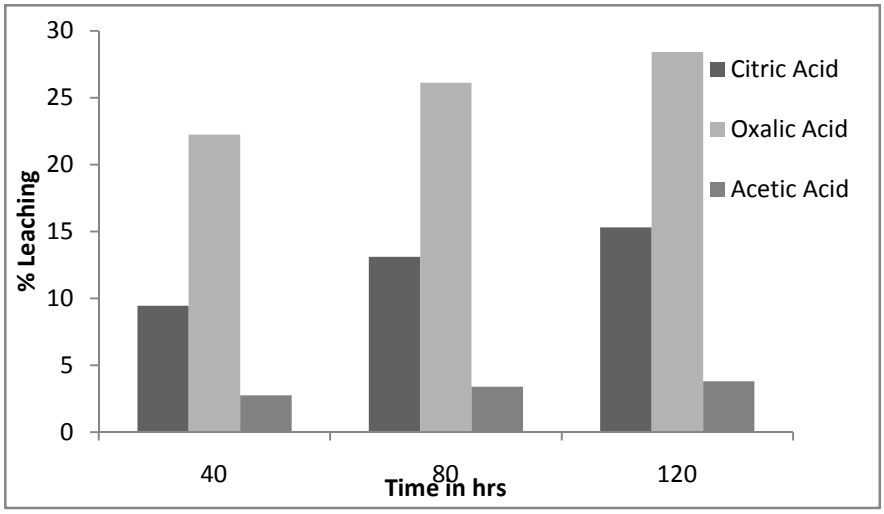

Figure 4.7 Effect of acid type on nickel leaching.

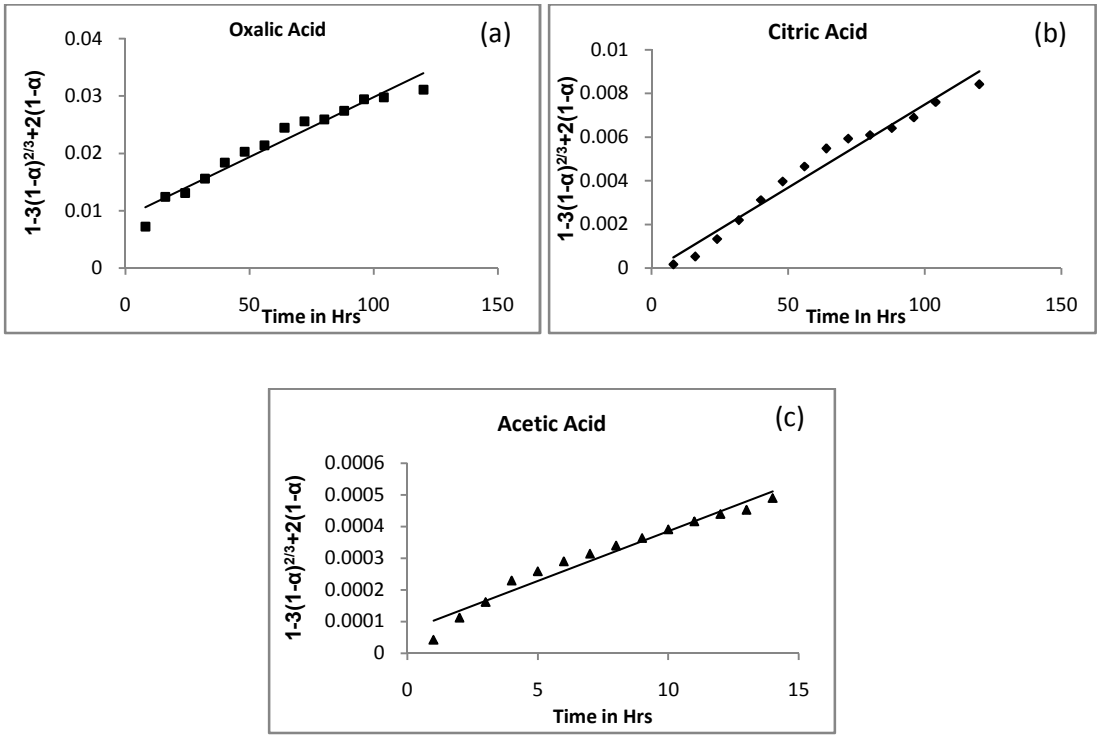

Figure 4.8 Plot of $(1-3(1-\alpha)^{2/3}+2(1-\alpha))$; ash layer diffusion controlled integral rate expression vs. time for (a) Oxalic acid (b) Citric acid (c) Acetic acid.

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Chapter 5

CONCLUSIONS AND FUTURE DIRECTIONS

In this chapter the salient accomplishments and major conclusions of this work are summarized and recommendations on future directions in the subject are made.

5.1. CONCLUSIONS

- An overview of the bio-mineral processing & the role of microbes in it, the present national and international state of art of the commercialization of *bio- mineral processing,* the lack of a smooth transition of technology from laboratory scale to commercial scale and the roadblocks of it have been documented here.
- For the three important metals; Fe, Ni, and Zn, an elaborate documentation has been presented with respect to their resources/reserves (both nationally and internationally), their ores, and uses.
- The potential of heterotrophs for leaching/ beneficiation are yet to be fully exploited along with its major challenges in process design remain unresolved. In this context, the characterization of a wide variety of heterotrophic micro organisms using iron ore beneficiation process as a case have been made and this study will contribute to the design database for bio-mineral processing using those heterotrophs.
- The application of 2^4 level of full factorial design of experiments for zinc bioleaching using autotrophs manifested the screening of design parameters along with optimum

parameter estimation. It also proposed the empirical model of the yield (zinc) as a function of major design parameters and the interactions among them.

- The characterization of secondary metabolite excreted by heterotrophs, proposition of efficient kinetic mechanism for laterite ore and secondary metabolite interaction (a heterogeneous one for nickel extraction) adapting the shrinking core model are the valuable excerpts of the present study.
- The design and execution of model driven experiments to establish the kinetics and generation of design parameter like effective diffusion coefficient of leaching acid through the ore body seems to be extremely useful for modeling simulation and control of this microbial process based on first principle. This will expedite the process of future scale-up of the two-tank leaching process of chromite mine overburden using heterotrophs.

5.2. FUTURE SCOPE AND DIRECTIONS

- In an ending note, this chapter concludes with recommendation of future research initiatives. Determination of activation energy of heterotrophic leaching of nickel laterite ore is an essential parameter for modeling this process on the basis of first principle. The quadratic curvature detected on the basis of the factorial analysis for zinc leaching demands a further investigation with a response surface model which may corroborate the estimated optimum influencing factors for zinc sulfide leaching using *Acidithiobacillus ferrooxidans.* Carrying out experiments at three levels may not only estimate the linear effects but also quadratic effects for maximal zinc recovery in the region defined by these diagnostic experiments.

PUBLICATIONS FROM THIS M-TECH. (Res.) WORK

Published & Communicated

- Sahu, S., Kavuri, N. C. and Kundu, M., (2009). Development of microbial process for bio-beneficiation of low grade iron ore using heterotrophic microorganisms. International symposium on waste, energy & environmental of mining & mineral based industries, IATES, Bhubaneswar, pp. 51-57.
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